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Engineered honey: In vitro antimicrobial activity of a novel topical wound care treatment

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ABSTRACT

Surgihoney is a novel engineered organic honey product for wound care. Its antimicrobial activity can be controlled and adjusted by the engineering process, allowing preparation of three different potencies, labelled Surgihoney 1–3. Susceptibility testing of a range of wound and ulcer bacterial isolates to Surgihoney by the disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination, and time–kill measurements by time suspension tests were performed. Surgihoney demonstrated highly potent inhibitory and cidal activity against a wide range of Gram-positive and Gram-negative bacteria and fungi. MICs/MBCs were significantly lower than concentrations likely to be achieved in topical clinical use. The topical concentration of Surgihoney in wounds was estimated at ca. 500 g/L. MICs/MBCs for *Staphylococcus aureus* were 32/125 g/L for Surgihoney 1 and 0.12/0.25 g/L for Surgihoney 3. Cidal speed depended on potency, being 48 h for Surgihoney 1 and 30 min for Surgihoney 3. Maintenance of the Surgihoney inoculum preparation for up to a week demonstrated complete cidal activity and no bacterial persistence. Surgihoney has wide potential as a highly active topical treatment combining the effects of the healing properties of honey with the potent antimicrobial activity of the engineered product for skin lesions, wounds, ulcers and cavities. It is highly active against multidrug-resistant bacteria. It is more active than other honeys tested and is comparable with chemical antiseptics in antimicrobial activity.

1. Introduction

Honey has been used for millennia as topical wound therapy based on observations of its healing properties and cleansing action on suppurating wounds [1–4]. This use of honey may have been based on further observations that honey in hives does not deteriorate or become contaminated [5,6]. The clinical burden of soft tissue damage is increasing. Superficial wounds and skin ulcers are becoming increasingly common with the rising age of the population in many countries and the global epidemic of obesity and type 2 diabetes [7]. In the UK, community nurses spend much of their time dressing leg ulcers, and supervision by leg ulcer nurses is essential if standards are to be maintained in community leg ulcer services [8]. Most chronic breaks in the skin become colonised with bacteria [9–11]. It is difficult to know when and whether these bacteria are pathogenic, but it is likely that even if overt infection is not present, bacterial colonisation plays a role in slowing tissue healing, allowing the establishment of biofilm and resulting in wound slough and an offensive odour [12,13].

Tissue viability is challenging, particularly when complicated by co-morbidities [14]. Chronic wounds always become colonised with bacteria, which may destabilise the healing process [9–13]. There is a temptation to send a microbiological sample and to offer systemic antibiotics when the sample is reported as growing bacteria. All this serves is to select ever more resistant microbes, which is why chronic lower extremity ulcers are so often colonised with multidrug-resistant (MDR) organisms such as meticillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* [15].

Surgihoney™ is a licensed sterile product that has been developed as a dressing for wounds. It consists of natural honey sourced from several sites that has been through a process to produce different potencies of antimicrobial activity which greatly exceed the activity of other honey dressings. It is comparable with chemical antisepsis but appears to retain the wound healing properties of natural honey [1–3].

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This study examined the in vitro properties of this novel engineered product based on natural honey that has been through a process which enhances its antimicrobial properties. Different grades of the product (Surgihoney 1–3) can be produced with increasing levels of antimicrobial potency. This level of antimicrobial activity can be replicated and is stable. Each of these Surgihoney grades may have a role in topical clinical treatment depending on the degree of antimicrobial activity required. This is an entirely novel process and product. As an engineered product, Surgihoney retains all of the established healing properties of natural honey, but its antimicrobial activity can be set at whichever potency is required. Surgihoney 1 is a sterilised, pharmaceutical grade product licensed for clinical use as a topical wound dressing in the UK. The other grades, 2 and 3, are currently prototype products. This study aimed to establish the in vitro efficacy of the Surgihoney grades against bacterial wound isolates by determining minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of Surgihoney 1, 2 as well as 3 and time–kill curves by suspension tests.

2. Materials and methods

2.1. Surgihoney

Surgihoney was provided by the manufacturer (Healing Honey Ltd., Bicester, UK) as potency grades 1, 2 and 3. The product grades were presented as a sterile product in a sachet in semisolid form.

2.2. Clinical isolates

Clinical isolates were collected from soft tissue microbiology samples submitted to the Microbiology Department of Hampshire Hospitals Foundation Trust (Winchester, UK). Eighteen isolates of *S. aureus* (12 meticillin-sensitive *S. aureus* and 6 MRSA), six isolates of β-haemolytic streptococci [Lancefield groups A (2), B (2), C (1) and G (1)], five isolates of *Enterococcus* spp. (including vancomycin-resistant *Enterococcus faecium*), six isolates of *Escherichia coli* [including extended-spectrum β-lactamase-producers], two *Klebsiella pneumoniae*, one *Serratia marcescens* AmpC-producer, four *P. aeruginosa*, one *Acinetobacter lwoffii*, one *Propionibacterium acnes*, one *Bacteroides fragilis*, two *Candida albicans*, one *Candida glabrata* and one *Aspergillus fumigatus* were tested against Surgihoney. Control NCTC strains were also tested.

2.3. Agar diffusion

Wells of 6 mm were cut in Iso-Sensitest agar (Oxoid Ltd., Basingstoke, UK) that had already been inoculated with the test organism at a concentration to give semiconfluent growth. Test samples of Surgihoney and other honeys in the pilot study were added to the wells.

A pilot study was carried out initially to compare Surgihoney potencies 1, 2 and 3 with a variety of honeys from around the world (Europe, South America, New Zealand, Yemen, Sudan) and with medical honey (Medihoney®; Comvita, Maidenhead, UK) as well as with antimicrobial dressings containing silver (Aquacel® Ag; ConvaTec, Deeside, UK) and iodine (Iodoflex™; Smith & Nephew, UK). Wells were cut in plates inoculated with *S. aureus* and were filled with test honey or, in the case of the dressings, these were cut to 2 cm × 2 cm and were placed on the surface of the inoculated plates.

Following the pilot studies, Surgihoney potencies 1, 2 and 3 were tested alone against a range of bacterial isolates from skin lesions. The wells were filled to the surface with a preparation of ca. 2 g of neat Surgihoney of the three potencies, diluted and emulsified in an equal volume of sterile water. Zone sizes were measured after 18–24 h of aerobic incubation (72 h for *Candida* and *Aspergillus* spp., and 18–24 h anaerobically for *Propionibacterium* and *Bacteroides* spp.).

2.4. Minimum inhibitory concentrations and minimum bactericidal concentrations

Surgihoney product was warmed to 37 °C to liquefy it and 5 g was mixed with 10 mL of sterile deionised water. This dilution was regarded as the 'neat' substance for serial dilution. The British Society for Antimicrobial Chemotherapy (BSAC) method for determining MICs and MBCs was used [16]. Surgihoney was serially diluted in 96-well microtitre trays. Starting with neat product, 75 μL of each honey dilution was added to the next well in the strip of the microtitre tray. The neat concentration represented a concentration of 256 g/L, and the 1:2048 dilution represented ca. 0.12 g/L.

Test organisms were prepared by taking four morphologically identical colonies for each organism from pure culture to create a 0.5 McFarland standard density. This was further diluted 1:10.

All wells, including controls, were inoculated with 75 μL of the test isolate preparation and the well trays were incubated at 37 °C for 18 h. The MIC was regarded as the most dilute well that showed no detectable turbidity.

The MIC well and those around the MIC well were subcultured on blood agar (Oxoid Ltd.) and were incubated at 37 °C for 18 h to determine the MBC. The MBC was the most dilute concentration that showed no growth after incubation.

Therapeutic concentrations of Surgihoney for comparison with the MIC and MBC were estimated by assuming that ca. 5–10 g of Surgihoney is applied to a wound. Local exudate will result in this being diluted in 5–10 mL of fluid. This will give an approximate local concentration of 500 g/L in contact with bacteria in the wound.

2.5. Time–kill curves

The test organism inoculum was prepared by taking 0.1 mL of a 0.5 McFarland standard density of the test organism and inoculating this in 4 mL of nutrient broth. The test inoculum was divided into four separate bijous, a control and three test preparations to which were added 0.5 g of Surgihoney 1, Surgihoney 3 or Medihoney. Colony counts of the inocula were determined by serial dilution 1:10 and plating 0.1 mL on a blood agar plate, repeated three times.

The test and control inocula were kept at 30 °C to simulate the temperature of a superficial skin lesion. Colony counting was performed as above in triplicate at 0.5, 2, 4, 24, 48, 72 and 168 h.

A terminal culture was performed by inoculating 0.1 mL of the original inoculum into nutrient broth to neutralise any residual effect of the Surgihoney and incubating for 72 h at 37 °C, before plating on blood agar to determine test organism survival.

3. Results

3.1. Inhibition zone sizes

The pilot comparative studies demonstrated that all of the Surgihoney potencies had greater antimicrobial activity than any other honey tested, including the medical grade honey, Medihoney. The inhibitory zones for Surgihoney 1, 2 and 3 were larger than those produced by any other honey. Silver dressings produced some inhibitory effect beneath the dressing but there was no zone of inhibition as there was for Surgihoney. Iodine dressings produced a large zone of inhibition (ca. 70 mm) to *S. aureus*,