A Comparison Between Medical Grade Honey and Table Honeys in Relation to Antimicrobial Efficacy

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Abstract: When antimicrobial agents are being evaluated prior to their introduction into clinical practice, advance publicity may interest potential users but access to that agent will normally be limited until licensed products are released and distributed. Honey is an ancient therapy that has recently been re-introduced into modern medicine. Medical grade honey (MGH) is being incorporated into sterile devices that are applied topically to wounds. Honey is universally recognized and it is readily accessible. Patients and practitioners may, therefore, consider using table honey from supermarkets as a cheap, readily available alternative to more expensive, regulated, honey-based wound care products. This study was designed to compare the antibacterial potency and microbial flora of 18 table honeys to a representative sample of Leptospermum honey (a MGH). Standardized tests of microbial content and in-vitro efficacy were conducted for each sample. Table honeys generally possessed lower antibacterial activity than the MGH and contained a wide range of microbial species, whereas MGH was sterile. The disadvantages of using non-sterile table honeys in medical practice were reviewed. Results suggest the need for randomized clinical trials verifying the efficacy and/or safety of any form of honey used in topical wound care.

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The development of a new antibiotic usually takes between 10 and 15 years and costs nearly $1 billion. During that time, a code number or trivial name that is meaningless to those not involved in its development identifies the agent. Although preliminary reports of its efficacy may excite potential
patients, their access to the new treatment will be restricted until the launch and distribution of the fully developed and licensed product. Less expensive alternatives may not be available immediately since the bioactive component(s) may be patent protected. Honey is a broad-spectrum antimicrobial agent that has been used in treating wounds for thousands of years. It has been shown to inhibit the growth of a wide range of bacteria, fungi, protozoa, and viruses.\textsuperscript{1,2} Laboratory tests with medical grade honeys (MGH), particularly Leptospermum honeys such as manuka or jellybush, have demonstrated that antibiotic-sensitive bacteria and antibiotic-resistant bacteria are equally susceptible to dilute concentrations.\textsuperscript{3–6} An increasing number of reports of the eradication of methicillin-resistant Staphylococcus aureus (MRSA) from wounds illustrate the clinical efficacy of such honeys.\textsuperscript{7–11} All honeys have a high sugar content, low water content, and acidity that prevents microbial growth. Most unprocessed honeys, when diluted slowly, generate hydrogen peroxide due to the activation of the enzyme glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide.\textsuperscript{12,13} Hydrogen peroxide has antibacterial properties, but not all honeys exhibit equal activity.\textsuperscript{1} In many honeys, heating at elevated temperatures destroys this peroxide activity and it is lost in the presence of catalase (an enzyme that degrades hydrogen peroxide and is present in wound fluid). However, Leptospermum honeys retain activity in the presence of catalase and are known as non-peroxide honeys.\textsuperscript{1} Contributions to the non-peroxide activity of Leptospermum honey by several components have been identified,\textsuperscript{14–17} but it is most likely that more have yet to be characterized. An in-vitro bioassay using S aureus allows the estimation of the antibacterial potency of honeys in relation to a solution of phenol.\textsuperscript{18} Total activity (peroxide and non-peroxide) is estimated by dilution of honey in water; non-peroxide activity is estimated by dilution in catalase. Honey largely disappeared from conventional medical practice during the 1970s,\textsuperscript{19} but was recently reintroduced into modern wound care through the development of sterile devices utilizing MGH. In Australia, the Therapeutic Goods Association licensed sterile MGH for wound care in 1999; the first MGH-impregnated dressing was licensed in the United Kingdom in 2004. A range of products is currently available throughout Europe and Australasia, and some are available in Canada and the United States. Medical journal studies reporting that difficult wounds treated successfully with prototype wound care products\textsuperscript{7,8,20,21} generated significant media and public interest in honey as a wound therapy in the United Kingdom, which prompted prospective patients to apply non-sterile honeys obtained from supermarkets and health stores to their chronic wounds. To date, no adverse events are known to have resulted from this manner of use, however, the possible presence of clostridial spores in such products cannot be ignored.\textsuperscript{22} While MGH is selected and processed to minimize adverse clinical events,\textsuperscript{23} honey intended for culinary use is not. This study was, therefore, designed to investigate the antibacterial potency and microbial flora of a selection of table honeys and one MGH to explore their therapeutic potential and safety.

Materials and Methods

Honey samples. During the spring of 2007, 17 samples of table honey were purchased from British supermarkets and one was purchased from a market stall in rural France (Table 1). Sample 19 was sterile, medical-grade manuka honey (Manukacare 18+™, Comvita UK, Ltd, Berkshire). This honey is marketed in Canada and the United States as Medihoney™ (Derma Sciences, Princeton, NJ). An artificial honey solution was used to determine whether inhibitory effects were due to the sugar content of the honey samples—100 g was prepared by dissolving 1.5-g sucrose, 7.5-g maltose, 40.5-g D-fructose, and 33.5-g D-glucose in 17-mL of sterile, deionized water. Honey samples were stored in the dark at 4˚C until tested (all tests were completed within 6 months of purchase). Each sample was mixed thoroughly with a sterile spatula to remove a representative sample for each test. Determination of antibacterial activity. The Allen et al\textsuperscript{18} bioassay was utilized to determine total and non-peroxide activity of all honey samples, except that the test organism used was S aureus NCTC 6571 instead of S aureus 1,2,3–6,7–11,12,13,14–17,18
ATCC 9144. Each sample was assayed in quadruplicate, and dilutions were prepared either with deionized water (to determine total antibacterial activity) or with 2 mg/mL catalase solution ([Sigma, Poole, UK] to determine non-peroxide antibacterial activity). Zones of inhibition were measured and compared to those obtained using reference solutions of phenol (Fisher Scientific, Fair Lawn, NJ), so that activity was expressed as phenol equivalent % (w/v). The inhibitory potential of each honey sample was estimated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against 6 bacterial cultures. Minimum inhibitory concentration. Using aseptic technique, a stock solution of each honey (1 g/mL) was prepared by weighing 10 g and making up to 10 mL in a volumetric flask using sterile iso-sensitest broth ([ISB], Oxoid, Cambridge, UK). Each stock solution was used to prepare a serial doubling dilution series to 31.25 mg/mL using iso-sensitest broth and 200-µL aliquots were transferred to a 96 well microtiter plate. Apart from the wells in column 1 (negative control: ISB only) and in row A (honey sterility control), all wells were inoculated with 1-µL of an overnight culture of test organism (approx. 106 cfu/mL). The following organisms were used: S. aureus NCTC 6571 (Oxford Staph), Escherichia coli NCTC 10418, E-MRSA 15 NCTC 13142, Staphylococcus epidermidis (clinical isolate), Streptococcus pyogenes (clinical isolate) and Pseudomonas aeruginosa (clinical isolate). Wells in column 2 were positive controls (no honey). Plates were incubated overnight at 37°C and absorbance determined at 490 nm using a plate reader (Dynex, West Sussex, UK). Bacterial growth (turbidity) was determined visually and confirmed with the plate reader printouts. The lowest concentration of honey to prevent growth of each organism was recorded as the MIC. All wells with no apparent growth were streaked to nutrient agar plates (Oxoid, Cambridge, UK) and incubated at 37°C for 24 hours to determine whether viable bacteria had persisted. The MBC was recorded as the lowest concentration of honey to prevent the survival of viable bacteria. These tests were repeated with honey samples prepared with ISB containing 2-mg/mL catalase.

Characterization of microbial flora of the natural honey samples. With the exception of the artificial honey sample, solutions of each honey were prepared according to the details provided in Table 2 and either plated directly onto the media specified or cultured (enriched) in broth and streaked onto selective media. Hence, the presence and number of mesophilic aerobic bacteria, mesophilic anaerobic bacteria, coliforms, salmonellae, clostridia, yeasts, and fungi were determined. Representative colonies were identified as far as possible using rapid identification kits for Gram-positive bacteria, Gram-negative bacteria, and anaerobes supplied by BBL and bioMérieux according to the manufacturers’ instructions.

Results

Using the bioassay, antibacterial activity was detected in 9 of the honey samples, with total activity ranging from 4.2% to 18.8% (w/v) phenol equivalent, and non-peroxide activity varying from 4.8% to 18.6% (w/v) phenol equivalent (Table 3). Five of the active honey samples (lavender, eucalyptus, tropical honey, and 2 heather samples) had no detectable non-peroxide activity, suggesting that they were hydrogen peroxide generating honeys. The remaining 4 active samples possessed non-peroxide activity, of which 1 sample was marketed as buckwheat and 3 samples were manuka (Leptospermum) honey. Nonperoxide activity has not been reported in buckwheat honey before and it is not known whether this particular sample is representative of all buckwheat honeys. It is possible that it was not a unifloral honey, but rather derived from nectar collected from several floral sources.

The labeling on the packaging of the manuka (Leptospermum) honeys (7, 15, and 19) claimed non-peroxide activity equivalent to 10%, 10%, and 18% (w/v) phenol equivalent, respectively. However, only sample 19 (the MGH) conformed to the label; the others possessed lower activity than stated (6.1 and 5.5 for samples 7 and 15, respectively). The capacity of antimicrobial agents to inhibit pathogenic organisms has long been evaluated in vitro using serial dilution in broth to determine the lowest
concentration able to prevent growth (MIC) and the lowest concentration to prevent the survival of viable bacteria (MBC). Here we tested two laboratory reference cultures (S aureus NCTC 6571 and E coli NCTC 10418) and 4 clinical isolates from chronic wounds (MRSA, S pyogenes, S epidermidis, and P aeruginosa). Artificial honey was included because its inhibitory action was confined to its sugar content and allowed a second way to identify inactive honeys. Samples 1, 3, 4, and 16 gave MIC values similar to artificial honey (Table 4), hence, 15 samples demonstrated varying levels of activity; sample 19 possessed the most effective inhibition. Demonstrating the survival of live bacterial cells in tubes after the MIC test, one can deduce whether an antimicrobial agent is bacteriostatic (prevents growth) or bactericidal (prevents survival). If the ratio of MBC to MIC is ≤ 4, then a bactericidal mode of action is indicated. In most cases, bactericidal action was found. Exceptions occurred with the lower-potency honeys (1, 3, 4, 6, 8, 9, 15, 16, 17, and artificial honey). The tests were limited because the maximum achievable honey concentration in the test was 1 g/mL. Again, MGH demonstrated greater activity and was bactericidal in 5 of the 6 test organisms. The MIC and MBC test results confirm that Gram-positive bacteria are more sensitive to honeys than Gram-negative bacteria and that the most susceptible test species was S pyogenes.

When the MIC and MBC were determined in the presence of catalase, only non-peroxide honeys (7, 15, 18, and 19) retained antibacterial activity (Tables 6 and 7). The MGH possessed the greatest extent of bactericidal action against the test organisms, while the peroxide honeys demonstrated potency similar to the artificial honey (Table 5). A wide variety of mesophilic aerobic bacteria were recovered from the 18 culinary honeys (Table 8). Many of these microorganisms are commonly associated with soil and are not normally considered pathogens. Some, however, may colonize chronic wounds such as Clostridium ramosum (honeys 6 and 14) and Staphylococcus warneri (honey 17). Bacillus species were most frequently recovered and were found in 14 honey samples.

Coliforms and salmonellae were not detected in the enrichment experiments with MacConkey broth and selenite broth. Although the numbers of anaerobes were shown to be low by plating onto blood agar, enrichment in Robertson’s cooked meat medium demonstrated their presence in 18 samples. Fusobacterium species was found in 9 samples and 3 samples contained clostridia. It was not possible to identify all of the anaerobic isolates with the BBL kit because it is designed for use with clinical, rather than environmental, bacteria. No organisms were detected in sample 19 (MGH). This was expected since the sample was irradiated and microorganisms should not have survived the sterilization process.

Discussion

Ancient physicians were selective in which honeys they included in their remedies. Variations in samples intended for wound care have been discussed, but comparative clinical studies to investigate differential healing characteristics have yet to be attempted. The data generated in this study clearly demonstrates the range of antibacterial activity associated with different honeys and illustrates the importance of selecting honey of high potency when the causative agents of wound infection are to be inhibited. Ten of the honey samples failed to demonstrate antibacterial activity in the bioassay, and of the 9 active honeys, the MGH tested here was a non-peroxide honey with the highest level of antibacterial activity. Peroxide generating honeys are likely to be inactivated in vivo since human cells and erythrocytes contain catalase, but Leptospermum honeys would not be inactivated because their non-peroxide antibacterial activity depends on a mixture of components other than hydrogen peroxide. Jars of Leptospermum honey labeled with inflated antibacterial potency and marketed to the public is an emerging problem in the United Kingdom. The purchase price of such honeys (manuka from New Zealand and jellybush from Australia) depends on non-peroxide antibacterial activity, which is normally tested in registered...
laboratories before being exported. Some traders seem to have misled the public. Honeys with proven antibacterial potency (10+) have been recommended for wound care preferentially over honeys of low or unknown potency. The quality of components used in the manufacture of licensed wound care products is assured by validated tests and regulated by authoritative bodies. This ensures the batch-to-batch consistency expected of medical products. Supermarket honey, however, does not undergo such rigorous controls. The therapeutic benefits of honey have been identified as the ability to stimulate rapid wound healing and the inhibition of wound pathogens. It seems logical to select honeys with high antibacterial potency for topical application to wounds rather than those at low or undetectable levels. Of the honeys tested here, the MGH was found to possess the greatest bactericidal activity. Clinical trials designed to evaluate the relative antimicrobial efficacy of different MGHs have not yet been executed.

The presence of microorganisms in raw honeys is not unexpected and routes for the contamination of honeys are understood. The sample with the highest count of mesophilic aerobic bacilli was sample 15, otherwise counts did not exceeded 1000 cfu/g. None of the honeys tested contained coliforms or salmonellae and Clostridium botulinum was not detected. The cohort of honeys tested here was small and most likely explains why Cl botulinum was not isolated. Prevalence of spores of Cl botulinum ranges between 2% and 24% of honeys and is the reason that honey is not fed to infants younger than 12 months. Wound botulism is rare and usually arises from the use of recreational drugs, but the possibility of acquiring this infection by the topical use of unsterile honey should not be dismissed. The aerobic spore-bearing bacterial species characterized were not overt pathogens, but some (Bacillus brevis, Bacillus coagulans, Bacillus licheniformis, Bacillus pumilus, and Bacillus subtilis) have been isolated on infrequent occasions from debilitated patients. One (Bacillus cereus) has been associated rather more frequently with infections such as abscesses, bacteremia, septicemia, burn cellulitis, osteomyelitis, and postoperative infections. Bacillus capillosus has been isolated from the intestinal contents of animals and birds. Honey is not normally collected from infected hives, but the isolation of Paenibacillus alvei from 4 samples (8, 9, 10, and 15) suggests that honey had been collected from unhealthy hives because this bacterium is often recovered from diseased honeybee larvae obtained from colonies infected with European foulbrood disease (EFB). One consequence of beehive infection is treatment with antibiotics and the contamination of honey collected from such treated hives. MGHs should therefore be screened to assure the absence of contaminating antibiotics. The presence of Paenibacillus alvei on plates caused some difficulties in the laboratory during the isolation of bacteria from honey samples as it swarmed across agar plates sometimes contaminating single colonies before they were subcultured to fresh plates. It is possible that species have not been successfully isolated because of this issue.

Conclusion

This study demonstrates the relatively low antibacterial activity of honeys available in British supermarkets and the presence of potentially pathogenic organisms in such honeys. To date, no infections have been traced to such sources, but the possibility of infecting vulnerable patients exists. While sterile, quality-assured wound care products that contain honey with proven non-peroxide antibacterial activity are available for clinical practice; the use of non-sterile honey samples cannot be justified. Both patients and practitioners should understand the limitations of self-medication with supermarket honeys and the benefits of proprietary wound care products.

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Author(s):
Interesting, but alas all bought honey was considered as “one group.” Should there have not been made a difference/comparison between non-adulterated and non-heated honey to just “any bottle named” honey? It has been accepted in Ayurveda medicine that heated honey is “toxic” [whatever that may mean in our Western terminology]. The fact that so many nutritional statements of Ayurveda have already been shown to be right would give this consideration extra weight I would think.
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