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Antimicrobial spectrum of honey for aerobic organisms as seen at the National Orthopaedic Hospital, Enugu

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Summary

Background: Honey is an ancient topical wound dressing that has evoked international interest. The widespread use of honey on wounds is being encouraged but differences in its antimicrobial spectrum exist. There are few local laboratory studies available to guide clinicians in this environment, hence this study.

Materials and methods: A sample of commercially prepared honey from the tropical bee species was obtained and used in this prospective study. The initial medium was a 1-in-3 dilution of pure honey-agar mixture. Against this was smeared isolates from aerobic cultures of wounds in the microbiology laboratory of the National Orthopaedic Hospital, and incubated at 37^0 C for 72 hours. Seventy-five samples are presented in this on going study, of which 67 are consecutive samples. The rest are specific isolates of pathogenic organisms grown from patients' wounds and cultured against the honey sample and incubated for 24hours. Eight such samples were further incubated up to 72 hours and checked. A crude flammability test for purity was applied to the honey sample before use.

Results: Honey was found to suppress the growth of common gram positive and negative organisms including multi-resistant Staphylococcus aureus. When the pure cultures were treated with honey for 72 hours and then streaked on agar plates, no aerobic organism grew thereafter, including those that had previously grown on the 1-in-3 honey-agar mixture.

Conclusion: Routine laboratory honey antimicrobial spectrum provided by use of honey-agar mixture is recommended as a guide to its clinical use.

Key words: honey, antimicrobial sensitivity, laboratory test.

Introduction

The use of honey as an antimicrobial and wound dressing agent is common in all continents.[1-3] Its efficacy has been proved in several difficult wounds, and widespread use of honey on wounds is being encouraged.[1-5] Clinical studies in Nigeria and beyond show there are differences in its spectrum of antimicrobial action.[1,3,6,7]. It would be important to note the sensitivity in different places which may serve as a guide to its clinical use.

Materials and methods

In the course of the study a number of honey samples were obtained but were found to be significantly contaminated with micro-organisms. Their antimicrobial spectrum was not investigated. Only one uncontaminated sample had its spectrum tested throughout the duration of this prospective study: Commercially prepared honey from the tropical bee species. It was kept sealed in a clean bottle on the shelf at room temperature, and used when needed. No sterilization technique was employed on the sample.

Initially a random selection of common pathogenic organisms was cultured onto a 1-in-3 dilution of pure honey/blood-agar base mixture, honey being added to the agar at 50^0C and the mixture left to cool to room temperature. Bacterial isolates sub cultured from blood agar cultures of wounds was smeared against this in the microbiology laboratory of the National Orthopaedic Hospital, and incubated at 37^0 C for 72 hours. The isolates were species of Staphylococcal, Coliform, and Pseudomonas organisms.

Following the clinical evidence of inhibition of bacterial growth, 1-in-3 honey-agar mixture was now used routinely against organisms obtained from wound swabs of patients of the hospital undergoing aerobic antimicrobial culture and sensitivity studies. The wounds were following burns and other traumatic wound infection. Seventy-five samples are presented in this on going study, of which 67 are consecutive samples. After 24 hours incubation this second set was examined for suppression of the sub cultured organisms. The antibiotic sensitivity profiles of the organisms from the first 18 swabs were simultaneously tested, and recorded. Five of these 18 were from burn wounds, 12 from wound infection and one unspecified. The organisms were

Pseudomonas, E. coli, Klebsiella, other Coliforms, Streptococci, Proteus, and Staphylococcus aureus.

The rest of the tests used specific isolates of pathogenic organisms grown from patients' wounds and cultured against the honey sample. This was done to increase contact time with honey. Five bacterial isolates of patients' wounds from blood agar medium was inoculated with pure honey and then streaked unto agar plates. They were incubated at 37^0C and examined after 24 hours and 72 hours consecutively for growth of the organisms. The isolates were Klebsiella (two), other Coliforms (two), and Staphylococcus aureus (one).

In the last group the sub cultured organisms were inoculated into honey for 24 hours and 72 hours respectively before streaking them on agar plate. They were again incubated under the same conditions and examined for growth. The first honey/blood-agar base preparation and sampling was done and examined by the last author while the rest were by the second author.

A crude flammability test was applied to the honey sample, as well as to a sample that grew organisms. A drop of honey was obtained by wire loop and held over a Bunsen burner flame. Close observation for a transient golden flash was made. It was noted only in the test sample and not in that which grew organisms.

Results

The first was a random sampling against isolates of Pseudomonas, Coliform, and Staphylococcal organisms. The 1 in 3 honey/blood-agar base mixture suppressed all organisms except Staphylococcus albus.

The next group consisted of 18 consecutive samples of in patients. Five were from burn wounds and 12 from wound infections. The swabs were initially cultured unto blood agar plate and the positive cultures sub cultured in 1-in-3 honey-agar plate. Four of the swabs had mixed growths. Two organisms grew while 20 were either suppressed or killed. This corresponded to 91% success. The Staphylococcus aureus isolates grown from burn wounds that were suppressed by the honey-agar mixture were resistant to cephtazidime, ceftriaxone, ciprofloxacin, pefloxacin, gentamicin, ampicillin, cloxacillin, amoxicillin, and erythromycin.

The third group consisted of 49 swabs from in-patients. Eleven of these grew no organisms on blood culture while two had mixed growths. The success rate on the honey/blood-agar base mixture was 74%. However of eight isolates of Klebsiella tested against honey, four grew. Of 15 isolates of other coliform organisms three grew. Of 23 isolates of Staphylococcus aureus, four grew. Every other aerobic organism isolated was inhibited or killed after 24 hours incubation.

The fourth group were isolates of organisms that were not always suppressed by the honey-agar mixture i.e. klebsiella, other coliforms, and Staphylococcus aureus. Growth of the latter two was noted after 24 hours but after 72 hours no growth was evident. The fifth group were also isolates of Klebsiella, other Coliforms, and Staphylococcus aureus. Growth of the first and last was noted after 24 hours, but after 72 hours again no growth was evident.

Discussion

Honey has been noted to have significant wound healing properties even when laboratory evidence of antimicrobial action was lacking.[1] There are different methods reported in in-vitro honey sensitivity tests.[1, 3, 6-8] The methodology in this study differs from some other studies, but follows the same principle of exposure of micro-organisms to honey dilutions in a reproducible manner. Its simplicity should ensure it can be reproduced by any laboratory involved in wound

cultures. However adding honey to agar at 50^0C may have led to some diminution in antimicrobial activity as honey is heat labile.[9] Whilst in-vitro evidence of 1-in-10 dilution of honey as an antimicrobial exists,[3] in-vitro Nigerian studies have cited 40%-50% concentration as effective, and 20%-30% ineffective against bacteria.[6, 7] This study used 33% effectively.

The isolates for this study came from chronic wounds, a number of which had become resistant to several antimicrobials. The study agrees with other studies that honey is effective against multi antibiotic resistant organisms.[2, 10] The antimicrobial effect of honey has been attributed to several properties. Its hypertonicity leads to desiccation and subsequent destruction of micro-organisms.[11] Honey contains inhibines as hydrogen peroxide[8] flavonoids and phenolic acid.[12] Gluconic acid and tetracyclines have also been extracted from it. [13] However not all honey samples contain all these substances,[8] which may in part explain the differences in the efficacy of different honey samples.

This study clearly showed that whilst honey had in-vitro antimicrobial action against aerobic organisms, it did not inhibit all isolates of the same organism taken from different wounds. This has been reported previously[3] and indicates the need for routine sensitivity studies (as with systemic antibiotics) before honey application. The findings of occasional resistance in differing isolates of the same organism, and complete inhibition for previously resistant organisms following prior inoculation with pure honey raise important issues. This finding of occasional resistance in differing isolates of the same organism followed 24 hour incubation. After 72 hours no organism was grown. In a previous study following 48 hour incubation no tested organism grew.[6] Perhaps those organisms would not have grown in our series if they were all observed at 48 or 72 hours after incubation. However as different sensitivity patterns exist for differing isolates of the same organism even in antibiotics both in-vitro and in-vivo, this may not have been as a result of the methodology. It may explain the occasional ineffectiveness of honey dressings in our environment. This finding appears to support the decision of some authors who combine honey with antibiotics.

Synergy for Pseudomonads has been reported. [2, 14] It may also indicate the need for more frequent dressings in wounds infected by such initially resistant organisms. Twice daily dressing with honey has been reported as effective by some workers, [2, 15] but in such wounds infected by initially resistant organisms, especially those highly exuding wounds that quickly dilute the concentration of honey on the wound surface, four to six hourly dressings may be necessary to get good results. More sensitive organisms will still be inhibited by less concentrated honey though diluted by tissue fluid; higher local concentrations of honey are important for less sensitive isolates as indicated by the study. Routine laboratory sensitivity tests for honey will help the clinician decide which wound infection will require such treatment. The rate of improvement in such wounds may also be slower when treated with honey alone.

Contamination of honey samples by organisms has been attributed to a number of factors, most especially recent contamination from handlers and containers. [16] If such samples are applied on wounds, especially in patients with local or systemic immuno-compromise, exacerbation of wound infection may result. Gamma-irradiation sterilizes honey without causing it to lose antimicrobial qualities [9] and is recommended for such samples. The purity of commercial honey samples obtained locally is questionable. Some unscrupulous traders sell boiled sugars in its place. These do not have the antimicrobial action of honey. [7] This not only strengthens the need for routine laboratory sensitivity tests, but also raises the question of a simple reliable test for pure honey. We do not know what was responsible for the golden yellow flash. The place of the flammability test needs to be determined.

Conclusion

Routine laboratory testing of honey before its topical application as an antimicrobial agent is advocated.

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