Anti-Staphylococcus aureus efficacy of six natural honey samples originated from Syria

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RESEARCH ARTICLE

Anti-Staphylococcus aureus efficacy of six natural honey samples originated from Syria

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ABSTRACT:
Honey possesses many beneficial effects including antimicrobial properties which have drawn considerable interest. Six types of natural honey originating from Syria were randomly selected and tested for their antimicrobial effects against Staphylococcus aureus isolated from wound swabs. All the six types of honey showed antibacterial activity, however, Nagilla sativa honey sample was the most potent with a MIC of 25% (w/v%). Results of this study indicate that the antibacterial activity of locally available honey varies depending on their floral source. Further work is needed to identify factors which determine the antimicrobial efficacy of these different honey types. Their good antibacterial potency could potentially be explored in vivo for the treatment of wound infections in patients.

KEYWORDS: Honey, Staphylococcus aureus, MIC.

INTRODUCTION:
Staphylococcus aureus is one of the prominent causes of skin and mucous membrane infections. It is non-motile facultative anaerobes Gram-positive bacteria, characterized by individual cocci that divide in more than one plane to form grape-like clusters. They are characterized by being catalase-positive and oxidase-negative. Most S. aureus is divided into two groups: methicillin-resistant (MRSA) and methicillin-sensitive (MSSA); and treatments of these infections are significantly dependent on the pathogen's antibiotic resistance. A combination of aggressive antibiotic therapy and removal of the source of infection are central to the management of S. aureus infection. The appropriate antibiotic is determined by numerous factors, including the antibiotic susceptibility of the infecting organism, the source of infection, the presence of endocarditis and/or other metastatic sites of infection, and patient factors, including underlying comorbidities, concurrent medication, and antibiotic allergies. Over the past 10 years, MRSA has become resistant to even last resort antibiotics. The first vancomycin Resistant S. aureus infection was reported in USA in 2002 and the spread of vancomycin resistance worldwide is now inevitable, and could potentially result in a return to pre-antibiotic era. Hence, due to the importance of S. aureus infections and the increasing prevalence of antibiotic-resistant strains, this bacterium has become the most studied staphylococcal species.

Honey has been traditionally used as a wound dressing for thousands of years. Scientific researches have proved that the efficacy of honey is due to multiple bioactivities that work in concert to expedite the healing process.
There is good evidence that honey possess a broad-spectrum antibacterial efficacy in addition to its ability to stimulate the immune response thus promoting the growth of tissues for wound repair, suppress inflammation, and bring about rapid autolytic debridement. Natural honey contains carbohydrates, water and other minor but important ingredients such as aromatic acids and phenolic compounds which account for its medical and biological properties for treating infections, burns, wounds and ulcers.

The increase in bacterial resistance to first line broad spectrum antibiotics and the significant decrease in the number of new antibiotics approved for market and the various complications involved with chronic wounds made honey an important natural alternative antibacterial agent and amain research material. Numerous reports and clinical studies have demonstrated the antimicrobial activity of honey against a broad range of Gram-positive and Gram-negative microorganisms, including multi-antibiotic resistant strains. The antifungal activity of the honey, especially anti-Candida activity has also been reported.

Data indicate that the beneficial effect of honey varies greatly depending on the floral source, geographical changes and processing. This has stimulated the search for different types of honey with antibacterial activity.

Till date Syrian honeys have been used mostly as home remedy and few scientific researchers have dealt with its medicinal proprieties. In our previous research, we had proved that some types of Syrian honey possess an in-vitro anti-bacterial activity against Pseudomonas aeruginosa with a MIC ranging from 12.5 to 25%. The aim of the present study was to investigate the antibacterial activity of six different Syrian honeys against S. aureus depending on the floral source from which nectar honey has been collected.

MATERIALS AND METHODS:

Honey Preparation

*Nagilla sativa,* Carduoideae flowers, Anise (Pimpinellansium), Oak and wild flowers, Montana and Citrus honeys were purchased directly from beekeepers and used in this study. All honey samples were transferred into glass sterile containers and filtered with a sterile mesh to remove debris. They were first checked for purity by streaking on blood agar plates and incubated at 37°C for 24 hours. Sample that showed no contaminations were stored at refrigeration temperature prior to testing in order to prevent photo-degradation.

**Culture Preparation**

*Staphylococcus aureus* strains were isolated from swabs collected from a wide range of infected wounds routinely submitted to the department of medical microbiology at AL-Andalus University Hospital. Standard morphological assays and biochemical tests were carried out to confirm the identity of the organisms according to standard procedures. Strains of *S. aureus* were cultivated for 24h at 37°C on Muller Hinton agar plates. They were then diluted in sterile saline and the optical density was adjusted to that of tube 0.5 in McFarland scale, to standardize the inoculum at 1.5x10^5cfu/ml.

**Detection of the Minimum Inhibitory Concentration**

Samples were prepared aseptically and were handled protected from direct sunlight to prevent hydrogen peroxide degradation. Honey solutions were prepared immediately prior to testing by diluting honey to the concentrations ranging from 50% to 1.6% w/v in nutrient broth.

One hundred μl of 0.5 McFarland standardized bacterial suspension was added to 1900 μl of test honey. Control wells contained broth only (negative control) or bacteria and broth (positive control). Tubes were incubated at 37°C for 24 h, and then the optical density was recorded at 620 nm.

**RESULTS AND DISCUSSION:**

Previous studies showed that honeys inhibit Gram positive cocci isolated from infected wounds especially methicillin-resistant *Staphylococcus aureus* and strains of vancomycin-sensitive enterococci. Other studies demonstrated that honeys could inhibit all community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) within 24 h of culture. In most honeys the antibacterial activity is due to hydrogen peroxide, but much of this is inactivated by the enzyme catalase that is present in blood, serum, and wound tissue or by catalase from Staphylococci. Osmolarity due to the high sugar content of honey is also beneficial as it draws water out of bacterial cells. The lowest concentration of sugar known to prevent the growth of *S.aureus* has a water activity of 0.86. Acidity and the presence of various non-peroxide compounds derived from the pollen or the nectar of flowers are also of great importance in the antibacterial mechanism of honey and explain the variation in the antibacterial properties of honey linked with the floral source as well as geographical origin and which can vary 100-fold between different honeys.
Results presented in table 1 showed a striking similarity between the efficacies of different honey samples against the *S. aureus* isolate. Except for *N. sativa* honey, all honey samples were ineffective in inhibiting the growth of *Staphylococcus aureus* when used in concentrations less than 50%. Our results are in agreement with the results of Nzeako and Hamdi who have tested six different honey samples and found that the inhibition of *S. aureus* did not occur at honey concentrations less than 40% (wt/vol)\(^{29}\). Baltrusaityte et al. tested 38 multifloral honey samples and found that the inhibition effect considerably decreased when honey samples were diluted from 50 to 25%\(^{29}\).

In practice, when undiluted honey is applied to wounds, it is diluted by exudates and its antimicrobial activity at low concentrations is therefore, crucial. Therefore, for clinical use, honeys with high levels of antibacterial activity should be selected to maximize therapeutic effects\(^{30}\). In our study, *N. sativa* honey was found to be the most potent with a MIC of 25%. Those results are similar to that obtained for honey obtained from Dembia in North Gondar Zone in Ethiopia\(^{31}\) and close to that of Malaysian tualang honey which was proven to exhibit an anti-*Staphylococcus aureus* at a MIC 20%\(^{32}\).

*N. sativa* honey was previously found to have high sugar content and to be more acidic than other honey types\(^{33}\), this could explain its great efficacy in inhibiting the growth of *S. aureus*. In addition, dilution of honey to 25% will certainly reduce its osmolarity to a level that ceases to control infection\(^{34}\) thus it is expected that the antibacterial activity of honey is not only due to its osmolarity, but also due to other important factors that are present in the composition of honey and which depend to a great extent on the bees’ source of nectar, the location of the flowers and related weather conditions, the storage time and conditions, and the method of preservative treatment.

**CONCLUSION:**

As *Staphylococcus aureus* bacteria are important infectious agents in skin and wound lesions, it is therefore of clinical significance that all types of honey samples tested showed efficacy in inhibiting the growth of isolated *S. aureus*. The present study reveals that the efficacy of different types of honey against *S. aureus* was dependent on the type of honey and the concentration at which it was administered. It was proved that all the six honey samples tested against the pathogenic bacteria were effective in inhibiting the pathogenic bacteria, however, *Nagila sativa* honey was the most potent with a MIC of 25%. Further chemical analysis is needed to identify the factors which determine the antimicrobial efficacy of *N. sativa* honey and test their biological activities as it may represent an effective and less expensive approach for local wound cleaning of *S. aureus* infected wounds.

**REFERENCES:**


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**Table 1: Minimum inhibitory concentrations (% wt/vol)**

<table>
<thead>
<tr>
<th>Honey type</th>
<th>Honey concentration (% wt/vol)</th>
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<tbody>
<tr>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>Nagilla sativa</td>
<td>-</td>
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<tr>
<td>Carduoideae flowers</td>
<td>-</td>
</tr>
<tr>
<td>Anise (Pimpinella anisium)</td>
<td>-</td>
</tr>
<tr>
<td>Oak and wild flowers</td>
<td>-</td>
</tr>
<tr>
<td>Montana</td>
<td>-</td>
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<tr>
<td>Citrus</td>
<td>-</td>
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+: bacterial growth.  -: bacterial inhibition


