

# Evaluation of inhibitory effect of honey on some bacterial isolates

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## **Abstract**

**Background:** Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives.

**Objectives:** The aim of this study was to investigate the antimicrobial activity of honey sample from Basrah region against certain bacterial isolate.

**Method:** different concentrations (25.0%, 50.0%, 75.0% and 100.0%) of honey sample where checked for their antimicrobial activities, using some medically important microorganisms including *Escherichia coli*, *pseudomonas* spp. and *staphylococcus aureus*. The minimum inhibitory concentrations (MIC) of the honey sample were determined on the selected microorganisms by using broth dilution technique

**Result:** The sample of honey show inhibitory effect *in vitro* at 50%, 75% and 100%

concentration on the various investigated microorganism except at 50% concentration where no inhibition zone on *Staphylococcus aureus*. However, no effect was observed at concentration 25%. The MIC for *Escherichia coli*, *pseudomonas* spp. and *staphylococcus aureus* were 6.25mg/ml, 1.5mg/ml and 12.5 mg/ml respectively. **Conclusion:** The study shows that honey, like antibiotics, has certain organisms sensitive to it, and provide alternative therapy against cretin bacteria. And shown to have an antimicrobial action against a broad spectrum of bacteria ( both gram positive and gram negative bacteria).

**Key words:** *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, honey, antibiotics, sensitivity, antimicrobial.

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## **Introduction**

The antibacterial activity of honey was first recognized in 1892, by van Ketel <sup>(1)</sup>. Honey is produced from many sources, and its antimicrobial activity varies greatly with origin and processing <sup>(2)</sup>. Honey has been used as a medicine in many cultures for a long time <sup>(3)</sup>. It has been rediscovered by the medical profession and it is gaining acceptance as an antibacterial treatment of topical infections resulting from burns and wounds <sup>(4)</sup>. Numerous studies demonstrate that honey possesses antimicrobial activity <sup>(1,2,5)</sup>.

Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives <sup>(5)</sup>, it destroys and / or

inhibits the growth of some pathogenic vegetative microorganisms <sup>(6)</sup>. An antifungal action has also been observed for some yeasts and species of *Aspergillus* and *Penicillium* <sup>(3)</sup>, as well as all the common dermatophytes <sup>(7)</sup>.

Honey possesses inherent antimicrobial properties, some of which are due to high osmotic pressure/low water activity, in which the low water activity of honey is inhibitory to the growth of the majority of bacteria, and to many yeasts and moulds. When applied topically to wounds, osmosis would be expected to draw water from the wound into the honey, helping to dry the infected tissue and reduce bacterial growth. Even when diluted with water absorbed from wounds, honeys would be likely to retain a water activity sufficiently low to inhibit growth of most bacteria. Honey is mildly acidic, with a pH between 3.2 and 4.5, Gluconic acid is formed in honey when

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bees secrete the enzyme glucose oxidase, which catalyses the oxidation of glucose to gluconic acid, the low pH alone is inhibitory to many pathogenic bacteria and, in topical applications at least, could be sufficient to exert an inhibitory effect<sup>(8)</sup>.

Hydrogen peroxide, the end product of the glucose oxidase system and tetracycline derivatives have The antibacterial properties against pathogens<sup>(9)</sup>. Low concentrations of this known antiseptic are effective against infectious bacteria and can play a role in the wound healing mechanism<sup>(10)</sup> and in Stimulation and proliferation of peripheral blood lymphocytic and phagocytic activity<sup>(11)</sup>. Other factors, such as low protein content, high carbon to nitrogen ratio, low redox potential due to the high content of reducing sugars, viscosity/anaerobic environment and other chemical agents/phytochemicals are also likely to play some role in defining antibacterial activity of honey<sup>(12)</sup>. Furthermore, honey has been employed to shorten the duration of diarrhea in patients with bactericidal gastro-enteritis due to bacterial infection<sup>(13)</sup>. However, honey has other important beneficial characteristics that are less influenced by storage conditions<sup>(14)</sup>. The aim of this study was to investigate the antibacterial activity of honey sample from Basrah region against certain bacterial isolate.

#### **Material and methods**

##### **Honey samples**

The honey sample used in this study was collected from Basrah province / Iraq, (Almuftia region); it was collected in sterile container and checked for purity on blood agar plate by streaked on blood agar plate, and incubated overnight. The honey sample was diluted by physiological saline to 25.0%, 50.0%, 75% and the non diluted honey (100.0%) referred to as neat. The study done in Al Sader

teaching hospital/ College of medicine, it was carried out during the period from January 2009 to April 2009.

##### **Microorganisms**

*Staphylococcus aureus* , *Escherichia coli* and *Pseudomonas* spp. were obtained from the Al Sader teaching hospital laboratory as clinical isolates and maintained in blood and macconkey' s agar and sub cultured in Müller Hinton media.

##### **Antimicrobial susceptibility testing**

The disc diffusion technique was used as previously described by Kirby-Bauer<sup>(15)</sup>, using different types of antimicrobials. All isolates were inoculated into Müller-Hinton broth (in 10 ml) and incubated for 18 h to 24 h; the density was then adjusted to 0.5 McFarland standards at wave length 625 nm.

##### **Microbiological tests**

##### **Preparation of honey suspensions for the disc diffusion test**

The disc diffusion test was carried out as described by Mirsa and Helms *et al.*<sup>(16, 17)</sup>. Eight millimetre diameter-filter paper was saturated with 0.1 ml of each of the honey suspensions. The density of the isolates was the same as that used in the antimicrobial susceptibility testing of the various chemotherapeutic agents. All the tests were performed in triplicate.

##### **Minimum inhibitory concentration (Broth dilution method) against the isolated organisms**

The broth dilution technique was used to calculate the minimum inhibitory concentration (MIC) of the honey samples. The test was carried out as described by Heuvelink *et al.*<sup>(18)</sup>. A suspension of the organism was adjusted to  $1.5 \times 10^5$  organisms/ ml and further diluted to 1:200 in Müller Hinton broth. Five millilitres each of Müller Hinton broth was pipetted into ten sterile screws capped test tubes. A weight of 100

mg/ml of the honey was dissolved completely in the first tube. A serial dilution of honey, with a dilution factor of half was established. Tube number 10 served as a positive growth control containing Müller Hinton broth and bacterial inoculum only, and an additional tube containing broth only was used as a negative control. A volume of 0.1 ml of the bacterial suspension ( $7.5 \times 10^5$  organism/ml) was added to each tube. The tubes were incubated at 37°C for 18 h and visually examined for evidence of turbidity. The lowest concentration of honey in the series that inhibited the growth of the organism was taken to be the MIC, expressed in mg/ml.

### **Results**

Honey sample showed marked inhibition of growth on *Pseudomonas* spp., the maximum inhibition zone was shown at concentration of 100% as 23mm, which reduce to 10mm at 75% and 8mm at 50% concentration (Table 1). Also the table showed that *Escherichia coli* grow with inhibition zone at concentration of 100% as 22mm, and the inhibition zone reduce to 12mm at 75% and 8mm at 50% concentration. *Staphylococcus aureus* showed a little less inhibition zone with honey sample. These were 20mm at 100% and 11mm at 75% concentration, however, no effect was observed at concentrations 50% and 25% (Table 1).

**Table 1: Antibacterial activities of different concentrations of honey against microbial isolate.**

|                          | inhibition zone (diameter in mm) |                         |                         |
|--------------------------|----------------------------------|-------------------------|-------------------------|
| Concentrations % (mg/ml) | <i>Staphylococcus aureus</i>     | <i>Escherichia coli</i> | <i>Pseudomonas spp.</i> |
| 100%                     | 20                               | 22                      | 23                      |
| 75%                      | 11                               | 12                      | 10                      |
| 50%                      | 0                                | 8                       | 8                       |
| 25%                      | 0                                | 0                       | 0                       |

$\chi^2 = 1.8$

df = 2

P > 0.05

Table 2 shows the zone of inhibition on the net concentration of honey that produced a greater inhibition than tetracycline and gentamicin on *pseudomonas* spp. (23mm, 16mm and 0mm respectively), and on *Escherichia coli* (22mm, 18mm, 20mm respectively), Except for *staphylococcus aureus*, where the tetracycline produced similar inhibition of honey 20mm and 18mm to gentamicin.

Studies on the minimum inhibitory concentration (MIC) of the honey on the tested organisms showed that the MIC were demonstrated against *pseudomonas* spp. (1.5 mg/ml) and the MIC was exhibited against *Staphylococcus aureus* (12.5 mg/ml), while the MIC for *Escherichia coli* was equal to 6.25 mg/ml ( Table 3).

**Table 2: Antibacterial activities of net honey against certain bacterial isolate compared with Gentamicin and Tetracycline.**

| Organisms                        | inhibition zone (diameter in mm) |            |              |
|----------------------------------|----------------------------------|------------|--------------|
|                                  | Honey 100%                       | Gentamicin | Tetracycline |
| <i>Staphylococcus aureus</i>     | 20                               | 18         | 20           |
| <i>Escherichia coli</i>          | 22                               | 20         | 18           |
| <i>Pseudomonas spp.</i>          | 23                               | 16         | 0            |
| $X^2 = 7.24$ $df = 2$ $P < 0.01$ |                                  |            |              |

**Table 3: The minimum inhibitory concentration (MIC) of the honey against tested organisms.**

| Organisms                    | Minimum inhibitory concentration (MIC) (mg/ml) |
|------------------------------|------------------------------------------------|
| <i>Staphylococcus aureus</i> | 12.5                                           |
| <i>Escherichia coli</i>      | 6.25                                           |
| <i>Pseudomonas spp.</i>      | 1.5                                            |

### Discussion

This study was undertaken to investigate *in vitro* antimicrobial activity of honey against certain bacterial isolates. In the study, honey sample showed the antimicrobial activity, and our result were in agreement with Willix et al. <sup>(19)</sup> who found that honey inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas sp.*, and also in agreement with Bilal <sup>(20)</sup> who found honey exhibited a fairly good antimicrobial activity against both Gram-negative and Gram-positive bacteria, and a remarkable activity was observed with *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The study showed antimicrobial activity against *Staphylococcus aureus*,

and this result agreed with Molan <sup>(21)</sup> who found the *Staphylococcus aureus*, is one of the bacterial species most susceptible to the antibacterial activity of honey. These might be due to the osmotic effect, the effect of pH and the sensitivity of these organisms to hydrogen peroxide, which represented an 'inhibine', factor in honey <sup>(22)</sup>.

The potency of neat honey (100% concentration) was found to be superior against all bacteria tested, and the best antimicrobial activity of honey occurs with *pseudomonas sp.* followed by *Escherichia coli*, these results of the study were in agreement with Adeleke et al. <sup>(23)</sup> where it showed an evident of increase in the percentage levels of bacterial sensitivity - as high as 100% for *P. aeruginosa* and 96.4% for *E.*

*coli*. Also of interest is the finding that the activity of gentamicin, both 4.0 and 8.0 µg/ml, was found to be virtually lower than that of undiluted honey or any of its aqueous dilutions.

And these results were corresponding with Abd-El Aal <sup>(24)</sup> who showed that honey have a greater inhibitory effect on isolated gram-negative bacteria (*Pseudomonas aeruginosa*, *Enterobacter* spp., and *Klebsiella*). Also El-Sukhon et al. <sup>(25)</sup> showed that gram negative bacteria to be more sensitive to action of honey than Gram-positive bacteria.

Mundoï et al. <sup>(26)</sup> discovered that the antimicrobial activity of honey was more with *Pseudomonas* and *Acinetobacter* spp, both with resistance to some antibiotics like gentamicin, ceftriazone, amikacin and tobramycin than other bacteria tested.

The previous study of Subrahmanyam <sup>(27)</sup> showed that strains of *Pseudomonas aeruginosa* resistant to routinely used and higher antibiotics were sensitive to the antibacterial action of honey.

Taormina et al. <sup>(28)</sup> studied the antimicrobial effect of honey on gram negative bacteria and attributed it to the presence of factors as high content of tetracycline derivatives , hydrogen peroxide and powerful antioxidants, as also to a naturally low pH, which is unsuitable for bacterial growth, and to the presence of phenolic acids, lysozyme, and flavanoids.

The demonstration of MIC showed that the most susceptible microorganisms to the honey are *pseudomonas* spp. Cooper <sup>(29)</sup> has reported that manuka honey had MIC of less than 10% against 17 strains of *P. aeruginosa* from infected wounds, and honeys which have a MIC of 10% to 20%, can be expected to be effective in preventing growth of *Pseudomonas*, followed by *Escherichia coli* and *Staphylococcus aureus*. and these

results was accordance with Willix et al. <sup>(30)</sup> who found the MIC (minimum inhibitory concentration) of the honeys was found to ranged from 1.8% to 10.8% (v/v), indicating that the honeys had sufficient antibacterial potency to stop bacterial growth if diluted at least nine times, and up to 56 times in the presence of *Staphylococcus aureus* .

The high antibacterial effect of honey sample in the disc diffusion test and the low MIC may be attributable to the presence of glucose oxidase, which is activated by dilution in water resulting in the production of hydrogen peroxide which is toxic to bacteria <sup>(31)</sup>.

It was concluded that the result, the study shows that honey, like antibiotics, has certain organisms sensitive to it, and provide alternative therapy against cretin bacteria. And shown to have an antimicrobial action against a broad spectrum of bacteria (both gram positive and gram negative bacteria). These antimicrobial properties would warrant further studies on the clinical applications of honey against bacteria and other microorganisms.

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