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ORIGINAL ARTICLE

# Antibacterial Activity of Five Types of Egyptian Honey against Bacteria Isolated from Hospital and Community-Acquired Infections

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***Antimicrobial resistance, Honey, Antibacterial effect, MIC, MBC***

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## ABSTRACT

***Background:*** *Multidrug resistance is an increasing global problem. Hence, integration of modern and traditional medicine like honey could help in fighting such problem. Honey has been known to have several antimicrobial properties, it is important to get benefit from this natural antimicrobial agent, which is commercially available.* ***Objectives:*** *to determine the in vitro antibacterial (bactericidal and bacteriostatic) effect of honey against organisms isolated from different community (CA) and hospital-acquired (HA) infections and compare the antibacterial potency of five different types of honey that are commercially used.* ***Methodology:*** *The antibacterial potential of five types of honey (citrus, black seed, mountain, marjoram and clover honeys) was determined against bacterial isolates of different CA and HA-skin and subcutaneous infections using tube dilution method to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).* ***Results:*** *Marjoram honey has the least MIC and MBC for all isolates. All tested honeys showed varied bacteriostatic and bactericidal activities. MIC range was 6.25-50% v/v and MBC range was 12.5-100% v/v. No significant difference in honey effect between sensitive and resistant isolates.* ***Conclusion:*** *All tested honeys have bacteriostatic and bactericidal activities against different types of bacteria. Pharmacological standardization and clinical evaluation of the commercial honey effects are considered an essential demand to use honey as a medical trend for treating infection.*

## INTRODUCTION

For several decades, naturally sourced antimicrobial agents have been investigated to replace current pharmaceutical antibiotics to confront the increasing problem of multidrug resistance among bacteria[1]. While many products have been reported to have some antimicrobial activity, honey in particular appears to be a highly effective antimicrobial agent. Although honey was mentioned in the Talmud, both the old and new testaments of the Bible, and the Holy Quran, the formal discovery of the antibacterial properties of honey was recorded in 1892 by Dutch Scientist Van Ketel[2].

Honey is a super saturated solution or semi-solid product that is synthesized naturally from nectar of flower by honey bees[3]. Its antibacterial properties are multifactorial, so resistance to its effect is not highly probable. It has been shown that the properties of honey with its hyperosmolarity, low pH (3.5-5), and hydrogen peroxide contents, are effective in inhibiting bacterial growth and healing wounds[4]. The hyperosmolarity of honey may inhibit bacteria by drawing fluids from bacterial cell causing bacterial dehydration and death[5]. Also, presence of phenolic acid and flavonoids in honey have anti-oxidant antibacterial effects[4]. Other antibacterial components of honey include lysozymes[6], antimicrobial peptides such as bee defensin 1[7], and the flavonoid pinocembrin[8].

Honeys obtained from different geographical areas, have shown many therapeutic effects. Since the ancient Egyptians used honey as a general remedy for variable diseases (including bacterial diseases)[9], it was interesting to investigate the antibacterial potency (bacteriostatic/bactericidal) of five of the currently available Egyptian honeys.

## METHODOLOGY

**Subjects:**

This study was carried out in Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University, on 50 patients admitted during the period of the research (May 2016 to July 2017) to surgical intensive care, burn and surgical units in Tanta University Hospitals, in addition to patients coming to outpatient clinic of Surgery.

***Inclusion Criteria***

- Inpatient with post-operative wound infection.
- Outpatients with different pyogenic skin or subcutaneous infections not acquired from any hospital or health-care admission.

***Exclusion Criteria***

- Outpatients received antibiotics for the preceding 7 days.

**Methods:**

***Sampling:***

Pus samples were collected from drained abscesses or infected wounds using sterile syringes or swabs under complete aseptic conditions.

***Isolation and identification of the infecting organism***

All samples were cultured on blood and MacConkey's agar plates. All plates were incubated aerobically at 37°C for 24-48 h. The bacterial growth was identified by using the routine microbiological methods.

***Antibiotic sensitivity testing***

Antimicrobial susceptibility of the isolates was determined by modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates according to the Clinical and Laboratory Standard Institute (CLSI) guidelines[10]. Gram-negative organisms were tested against the following: imipenem (10 µg), amikacin (30 µg), ceftazidime (30µg), ciprofloxacin (5 µg), aztreonam (30 µg), amoxicillin/clavulanic acid (20/10 µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg), cefoxitin (30 µg), and colistin (10 µg).

While, Gram-positive organisms were tested against penicillin G (10 units), vancomycin (30 µg), linezolid (30µg), cefoxitin (30µg), sulfamethoxazole/ trimethoprim (1.25/23.75µg), erythromycin (15µg), ciprofloxacin (5 µg), and amoxicillin/clavulanic acid (20/10µg). *E. coli* (ATCC 25922),*Pseudomonas aeruginosa* (ATCC 27853), Methicillin-sensitive *Staphylococcus aureus* (MSSA) (ATCC 25923), Methicillin-resistant *S. aureus* (MRSA) (ATCC 43300) were used as control strains.

- ***Testing of antibacterial effects (MIC and MBC) of different honey types:***

Five Egyptian honey samples of different origins, namely, citrus, black seed, mountain, marjoram and clover honeys were obtained from the Ministry of Agriculture, Cairo, Egypt. All honey samples stored in the dark at room temperature until further use.

Few pure colonies from each bacterial isolate were picked up, suspended in 5 ml of nutrient broth and incubated aerobically, at 37ºC for 24 h. Then, the bacterial suspension was diluted with sterile distilled water until it matches the turbidity of 0.5 McFarland standard ($10^6$). The resulting suspensions were further diluted 1:100 in sterile nutrient broth to fix inoculums density of $10^4$ CFU/ml according to Wasihun *et al.*[11]

The minimum inhibitory concentration (MIC) of the honeys was measured by using tube dilution method according to Kacániová *et al.*[12] Then, tubes were incubated aerobically at 37 °C for 24 h and observed by visual inspections for the presence or absence of turbidity. All isolates with different species were tested to each of the honey types by the same method. To determine the minimum bactericidal concentration (MBC), incubated tubes showing no visible turbidity, were subcultured on sterile nutrient agar plates and incubated aerobically at 37 °C for 24 h. The least concentration of honey that did not show any growth of the tested isolates was considered as the MBC[12].

**Statistical analysis**

The results for quantitative variables were expressed as mean ± SD and were analyzed using Student's t test. Chi-square test was used to examine the relation between qualitative variables. A $P<0.05$ was considered statistically significant.

## RESULTS

**Distribution of Cases:**

This study included 50 patients with different skin and subcutaneous infections. Thirty patients had HA-infection (group I), while the other 20 patients had CA-infection (group II). Demographic characteristics of the included subjects are shown in (table 1). Among the included subjects, 56% were male and 44% were female and the age range was (1-70) years with the mean 44.3±17.6 years. There were no significant differences between the 2 groups according to age, gender or the associated diseases. Among the different clinical presentations, post-operative wound infection was the most frequent presentation (46%), followed by burn infection (18%), then abscess (12%), while the remaining infections had the same prevalence (6%). Abscess infection was significantly higher in community-acquired (CA) cases, while post-operative wound infection was significantly limited to hospital-acquired (HA) cases.

**Bacterial Outcome:**

Polymicrobial infection (i.e infection with more than one organism[13]) was detected in 10% of both HA- and CA-cases, while 50% of HA-cases and 75% of CA-cases showed monomicrobial infection. No bacterial growth was encountered in 40% of HA-cases and 15% of CA-cases. There was no significant difference between the two study groups according to the bacterial outcome. The 3 polymicrobial HA-cases were as follows, 2 of them were combined *E. coli* and *Candida* infections, and the remaining one was combined *E. coli* and *P. aeruginosa* infection. While the 2 polymicrobial CA-cases were a case of combined *E. coli* and coagulase-negative *Staphylococci* (CoNS), and a case of combined *E. coli* and *Proteus* (table 1).

**Table 1: Demographic characteristics of study population**

| Characteristics | Hospital-acquired (n=30) | Community- acquired (n=20) | Total (n=50) | *P*- value |
|---|---|---|---|---|
| **Age (in years)** | | | | |
| Mean ± S.D<br>Range | 43.8±18.3<br>1-66 | 45.1±16.9<br>3-70 | 44.3±17.6<br>1-70 | 0.803 |
| **Gender** | | | | |
| Male<br>Female | 19 (63.3%)<br>11 (36.7%) | 9 (45%)<br>11 (55.0%) | 28 (56 %)<br>22 (44%) | 0.201 |
| **Associated disease** | | | | |
| No associated disease<br>Hypertension<br>Diabetes<br>Hypertension+ Diabetes | 15 (50 %)<br>8 (26.7%)<br>6 (20%)<br>1 (3.3%) | 10 (50%)<br>3 (15%)<br>6 (30%)<br>1 (5%) | 25 (50%)<br>11 (22%)<br>12 (24%)<br>2 (4%) | 0.9 |
| **Clinical presentation** | | | | |
| Abscess | 0 (0%) | 6 (30%) | 6 (12%) | 0.037* |
| Burn | 5 (16.7%) | 4 (20%) | 9 (18 %) | 0.809 |
| Bedsore | 2 (6.7%) | 1 (5%) | 3 (6%) | 0.612 |
| Cellulitis | 0 (0%) | 3 (15%) | 3 (6%) | 0.116 |
| Paronychia | 0 (0%) | 3 (15%) | 3 (6%) | 0.116 |
| Post-operative wound | 23 (76.7%) | 0 (0%) | 23 (46%) | 0.001* |
| Traumatic wound | 0 (0 %) | 3 (15%) | 3 (6%) | 0.116 |
| **Bacterial outcome** | | | | |
| Polymicrobial<br>Monomicrobial<br>No growth | 3 (10%)<br>15 (50%)<br>12 (40%) | 2 (10%)<br>15 (75%)<br>3 (15%) | 5 (10%)<br>30 (60%)<br>15 (30%) | 0.135 |

***** Statistically significant

**Distribution of Organisms:**

Forty organisms were isolated from 50 included subjects, 21 organisms were isolated from HA-cases, and 19 organisms were isolated from CA-cases. Of the 40 isolated organisms, 26 were Gram-negative bacteria, 10 were Gram-positive bacteria and 4 isolates were fungi (table 2). Gram-positive bacteria were significantly higher in CA-cases, while both groups are statistically homogenous concerning Gram-negative isolates. The most frequent Gram-negative organism in all cases was *E.coli* (27.5%), followed by *P. aeruginosa* (15%), and then *Klebsiella*, *Acinetobacter* and *Proteus* (15% for each). MRSA was the most frequent Gram-positive isolates allover the study (17.5%), followed by MSSA (5%) and CoNS (2.5%) (table 2).

**Table 2: Different HA- and CA organisms isolated during the study**

| Organism | Hospital- acquired (n=21) | Community- acquired (n=19) | Total (n=40) | *P-value* |
|---|---|---|---|---|
| **Gram-negatives** | | | | |
| ***E. coli***<br>***Klebsiella***<br>***Pseudomonas***<br>***Acinetobacter***<br>***Proteus*** | 6 (28.5%)<br>2 (9.5%)<br>5 (23.8%)<br>3 (14.3%)<br>1 (4.8%) | 5 (26.3%)<br>1 (5.3%)<br>1 (5.3%)<br>0 (0%)<br>2 (10.5%) | 11 (27.5%)<br>3 (7.5%)<br>6 (15%)<br>3 (7.5%)<br>3 (7.5%) | 0.571 |
| **Gram-positives** | | | | |
| ***Staph aureus***<br>MRSA<br>MSSA<br>**CoNS** | 0 (0%)<br>0 (0%)<br>0 (0%)<br>1 (4.8%) | 9 (47.39%)<br>7 (36.8%)<br>2 (10.5%)<br>0 (0%) | 9(22.5%)<br>7 (17.5%)<br>2 (5%)<br>1 (2.5%) | 0.038* |
| **Fungi** | | | | |
| ***Candida*** | 3 (14.3%) | 1 (5.3%) | 4 (10%) | |
| **Total** | 21(100%) | 19(100%) | 40(100%) | |

***** Statistically significant, **MRSA**; methicillin-resistant *Staphylococcus aureus*, **MSSA**; methicillin-sensitive *Staphylococcus aureus*, **CoNS**; coagulase-negative *Staphylococci*

**Antibacterial Effect (MIC/MBC) of Tested Honeys:**

The MIC and MBC ranges of different honey types used against organisms isolated during the study period are defined in (table 3 and 4, respectively). Marjoram honey had the least MIC and MBC ranges for all isolates, while the other honey types showed nearly equal ranges. *E. coli* had the highest resistance (MIC and MBC) to all honey types compared with other tested organisms. Interestingly, MRSA, MSSA and *Acinetobacter* had the lowest resistance (MIC and MBC) among all tested organisms, especially to marjoram and mountain honeys. There were no significant differences in effect of honey between sensitive and resistant bacteria (data not shown).

**Table 3: Comparative MIC values of the five honey types on different organisms**

| MIC | | Mountain honey | Clover honey | black seed honey | Citrus honey | Marjoram honey |
|---|---|---|---|---|---|---|
| **Gram-negatives** | | | | | | |
| ***E. coli*** | | | | | | |
| **HA (n=6)** | **range** | 25-50 | 25-50 | 25-50 | 25-50 | 12.5- 25 |
| **CA (n=5)** | **range** | 25-50 | 25-50 | 25-50 | 25-50 | 12.5-25 |
| ***Klebsiella*** | | | | | | |
| **HA (n=2)** | **range** | 25 | 25 | 25-50 | 25-50 | 25 |
| **CA (n=1)** | **range** | 25 | 25 | 25 | 25 | 25 |
| ***Pseudomonas*** | | | | | | |
| **HA (n=5)** | **range** | 25-50 | 25 | 25-50 | 25 | 12.5-25 |
| **CA (n=1)** | **range** | 25 | 25 | 25 | 25 | 12.5 |
| ***Acinetobacter*** | | | | | | |
| **HA (n=3)** | **range** | 12.5 -25 | 12.5-25 | 12.5 -25 | 12.5 -25 | **6.25-12.5#** |
| ***Proteus*** | | | | | | |
| **HA (n=1)** | **range** | 25 | 12.5 | 25 | 25 | 12.5 |
| **CA (n=2)** | **range** | 25 | 12.5-25 | 25 | 12.5-25 | 12.5-25 |
| **Gram-positives** | | | | | | |
| **MRSA** | | | | | | |
| **CA (n=7)** | **range** | **6.25-12.5#** | 12.5-25 | 12.5-25 | 12.5-25 | **6.25-12.5#** |
| **MSSA** | | | | | | |
| **CA (n=2)** | **range** | 12.5-25 | 12.5-25 | 12.5-25 | 12.5-25 | **6.25-12.5#** |
| **CoNS** | | | | | | |
| **HA (n=1)** | **range** | 12.5 | 25 | 25 | 25 | 12.5 |

**MIC;** minimum inhibitory concentration, **HA;** hospital-acquired, **CA;** community-acquired, **MRSA**; methicillin-resistant *Staphylococcus aureus*, **MSSA**; methicillin-sensitive *Staphylococcus aureus*, **CoNS**; coagulase-negative *Staphylococci*, # Least MIC value

**Table 4: Comparative MBC values of the five honey types on different organisms**

| MBC | | Mountain honey | Clover honey | black seed honey | Citrus honey | Marjoram honey |
|---|---|---|---|---|---|---|
| **Gram-negatives** | | | | | | |
| ***E. coli*** | | | | | | |
| **HA (n=6)** | **range** | 50-100 | 50-100 | 50-100 | 50-100 | 25-50 |
| **CA (n=5)** | **range** | 50-100 | 50-100 | 50 | 50-100 | 25-50 |
| ***Klebsiella*** | | | | | | |
| **HA (n=2)** | **range** | 50 | 50 | 50-100 | 50-100 | 25-50 |
| **CA (n=1)** | **range** | 50 | 50 | 50 | 50 | 50 |
| ***Pseudomonas*** | | | | | | |
| **HA (n=5)** | **range** | 50 | 50 | 50 | 50-100 | 25 |
| **CA (n=1)** | **range** | 50 | 50 | 50 | 50 | 25 |
| ***Acinetobacter*** | | | | | | |
| **HA (n=3)** | **range** | 25 | 25 | 25 | 25 | **12.5-25#** |
| ***Proteus*** | | | | | | |
| **HA (n=1)** | **range** | 50 | 25 | 50 | 25 | 25 |
| **CA (n=2)** | **range** | 50-100 | 25-50 | 50 | 12.5-25 | 25 |
| **Gram-positives** | | | | | | |
| **MRSA** | | | | | | |
| **CA (n=7)** | **range** | 25-50 | 25-50 | 25-50 | 25-50 | **12.5-25#** |
| **MSSA** | | | | | | |
| **CA (n=2)** | **range** | 25-50 | 25-50 | 25-50 | 25-50 | **12.5-25#** |
| **CoNS** | | | | | | |
| **HA (n=1)** | **range** | 50 | 50 | 50 | 50 | 25 |

**MBC;** minimum bactericidal concentration, **HA;** hospital-acquired, **CA;** community-acquired, **MRSA**; methicillin-resistant *Staphylococcus aureus*, **MSSA**; methicillin-sensitive *Staphylococcus aureus*, **CoNS**; coagulase-negative *Staphylococci*, # Least MBC value

## DISCUSSION

The current study included 50 cases who had different skin or subcutaneous infections, only 35 cases showed microbial growth with an isolation rate of (70%). In agreement with this result, Muluye *et al.*[14] found also an isolation rate of (70.2%) among patients with pus and/or wound discharge. On the other hand, higher rate of isolation (87.3% and 83.9%) was detected by Mama *et al*.[15] and Mohammed *et al*.[16], respectively. On the other hand, lower rate of isolation was observed by Hailu *et al*.[17] who found an isolation rate of (61.6%). This disparity might be due to differences in wound discharge collection techniques, transport of samples, and number of cases. Another cause of disparity is that some patients might be under antibiotic treatment that affected bacterial growth.

In the present study, Gram-negative organisms were the commonest isolates in HA-infections, where *E. coli* was the most frequent isolates (28.5%), followed by *P. aeruginosa* (23.8%), then *Acinetobacter* and *Candida* (14.3%, for each). While, Gram-positives represented only (4.8%) of all HA-isolates. On the contrary, CA-infections in the current study resulted mainly from Gram-positive organisms, where MRSA were the commonest CA-isolates (36.8%), followed by *E. coli* (26.3%), then MSSA and *Proteus* species (10.5%).

These results more or less were correlated with the Egyptian study of Hafez *et al*.[18] who found that the most common isolates of HA surgical site infections were *Klebsiella* spp. (18%), *E. coli* (16.4%), and *P. aeruginosa* (16.4%), while, *S. aureus* and CoNS were accounted for only (6.6% for each) of the isolated pathogens. On the other hand, Mohammed *et al.*[16] reported that inpatient isolates were mainly *S. aureus* (23.7%), followed by *Klebsiella* (13.4%), then CoNS (11.4%), *Pseudomonas* (8.3%), *Proteus* (4.1%) and *E. coli* (3%). The high occurrence of Gram-negatives in this study may be due to contamination of wounds by enteric organisms during surgical procedure as majority of the operations were undertaken on abdomen. Also, Gram-negative bacteria became endemic in hospital environment as they easily transfer resisting common antiseptics[19].

The present study revealed that marjoram honey had the least MIC and MBC ranges against tested isolates. This result was in agreement with the Egyptian study conducted by Wasfi *et al*.[9] who found that marjoram honey showed the highest antibacterial activity against only one tested organism *E. coli*, as indicated by having the lowest MIC value as (25% w/v). Similarly, another Egyptian study found that honey from bees fed by marjoram were higher in their antimicrobial potency than honey from control colonies. Moreover, some studies reported the high antimicrobial activity of marjoram oil against both Gram-positive and negative bacteria[21,22].

On the contrary, another study found that mountain honey has strong inhibitory effect in comparison to other tested honey types, which were citrus, clover, and black seed honeys[23]. Other honey types tested in our study (clover, citrus and black seed honeys), had nearly equal MIC and MBC against all isolates. This was in agreement with Wasfi *et al*.[9] and Hassanein *et al*.[23]

This variability in results could be explained by the fact that different honeys vary in their antibacterial potency, which may be due to variations in plant source or the geographical distribution. Much of the literature that discussed the use of honey in treating microbial infections, did not clear the type of honey used. Other factors that may affect honey activity are seasonal changes, harvesting, processing, and storage conditions of the tested honeys. Therefore, honeys are not equal in their antimicrobial effectiveness [9,24].

The present study showed that *E. coli* was the highest resistant organisms to all tested honeys. This was in agreement with other studies who detected that *E. coli* was more resistant than other bacteria to the same tested honeys[25,26,27]. This resistance may be attributed to the lower permeability of the outer membrane of Gram-negative than Gram-positive bacteria that diminishes the entry of antimicrobial agents like honey into the bacterial cell[27].

On the contrary, another study reported that honey inhibited *E. coli* growth more than other tested bacteria[28]. These variations in bacterial resistance levels could be explained by various factors; since the antibacterial role of honey is attributed mainly to its high osmolarity, acidity, and hydrogen peroxide and non-peroxide contents, changes in the level of these factors lead to variations in overall antibacterial activity of honey[29].

## CONCLUSION

Antimicrobial resistant bacteria is a serious problem that endangers the public health. Therefore, we urgently need to develop alternative antibacterial strategies, and re-evaluate the therapeutic use of ancient remedies, such as honey. Conclusively, our results revealed that all tested honeys had both bacteriostatic and bactericidal activities. Marjoram honey exhibited the highest total antibacterial activity against all tested bacterial isolates.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**Ethical consideration**

It was approved by the Ethical Committee of the Faculty of Medicine, Tanta University, Egypt.

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