Antibacterial activity of Omani honey alone and in combination with gentamicin

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ABSTRACT

Objectives: To investigate the anti-*Staphylococcal* activity of Omani honey, gentamicin and combination of the 2.

Methods: This study was conducted in the Laboratories of the Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, Sultanate of Oman in 2004. Thirty honey samples from different parts of Oman were investigated for their activity against *Staphylococcus aureus*, using an agar well diffusion technique. The honey sample giving the best anti-*staphylococcal* activity was selected and further investigated for the killing rate on its own and in combination with gentamicin using tube dilution technique.

Results: Marked variations in the antibacterial activity of the different honey samples were observed. Thirteen of the Omani honey samples (43%) showed excellent anti *Staphylococcus aureus* activity. The best of the excellent honey samples (OH26), at a concentration of 50%, showed killing rate of 38% of *Staphylococcus aureus* at 30 minutes and 45% at one hour. Gentamicin (at 4 µg/ml) killed 70% and 88% while the rate of killing for the combination of honey and gentamicin was superior with 92% and 93% killing in the same duration.

Conclusion: Omani honey, in-vitro, possess anti-*Staphylococcus aureus* activity, which enhances gentamicin activity by 22% in the early phases of interaction.

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The therapeutic effects of honey has been well demonstrated and its antibacterial properties are well established.[1-4] Honey is known to have high therapeutic actions on different bacteria including those that are highly resistant to antibiotics.[5-9] Honey has been used as an adjuvant for accelerating wound healing from ancient times and has been sporadically used in treatment of burns.[2] Honey serves as an important medicine due to its mild laxative, bactericidal, sedative and antiseptic characteristics.[7] Honey is also known to act as highly viscous barrier preventing bacterial penetration and colonization of wound surface. [2]

Honey in combination with lemon, are traditional remedies in the Middle East and China and for many centuries have been used in treatment and prevention of common cold and treatment of various upper respiratory tract infection.[1] It is well established that certain antibiotics when combined, give superior synergic activity.[7] It is a common practice to combine beta-lactam antibiotics with aminoglycosides to achieve this objective. The objective of the present study is to investigate whether honey possesses synergistic or enhancement activity to an antibiotic.

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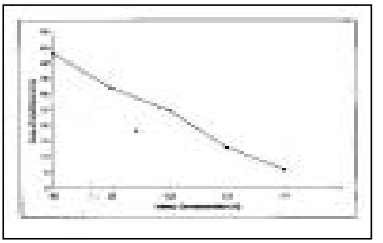

Figure 1 - Zones of inhibition (in mm) of the various dilutions of honey sample (OH26). OH26 - Omani honey number 26.

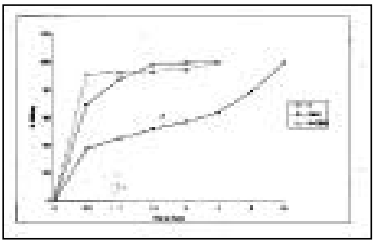

Figure 2 - Killing rate of *Staphylococcus aureus* using Omani honey, gentamicin and combination of the 2.

Table 1 - Anti-*Staphylococcus aureus* activities of the Omani honey samples.

Origin of the honey sample	Excellent	Good	Fair	Poor	Total
			N of honey samples		
South of Oman	3	6	2	0	**11**
Muscat	3	2	2	0	**7**
Al-Batinah	5*	1	1	1	**8**
Al-Dhahriah	0	1	0	1	**2**
Al-Wasta	2	0	0	0	**2**
Total	**13 (43%)**	**10 (33%)**	**5 (17%)**	**2 (7%)**	**30 (100%)**

*Honey sample (OH26), from Al-Batinah region, showed the best anti-*Staphylococcus aureus* activities.
Samples with zones of inhibition in diameter of: >30 mm (excellent), 21-29 mm (good),
10-20 mm (fair) and <10 mm were ranked as poor.

Table 2 - The *Staphylococcal aureus* killing rate (%) in honey alone, gentamicin alone and combination of the 2.

Substance	Percentage of killing rates							
	0 hour	0.5 hour	1 hour	1.5 hours	2 hours	4 hours	6 hours	24 hours
OH26 (50% concentration)	0	38	45	52	57	64	79	100
Gentamicin (4 µg/ml)	0	70	88	98	99.5	100	-	-
OH26 and gentamicin combination	0	92	93	94	95	100	-	-

OH26 - Omani honey sample number 26.

The antimicrobial activity of Omani honey has been investigated using honey samples from different parts of Oman against different bacteria.[8] It has been demonstrated that not all honey samples do possess antibacterial activity, though some showed excellent activity, while others failed to show any detectable antibacterial activity.[8] Moreover, most samples from Oman showed anti-*Staphylococcus aureus* activity.

Methods. Thirty different honey samples from different parts of Oman (OH1-OH30) were collected shortly, after harvesting, and stored in sterile universal containers and kept at room temperature (22-26 °C) for no more than 2 months, before testing. Each sample of honey was double diluted in series of 50%, 25%, 12.5% and 6.3% of its original concentration with sterile distilled water. *Staphylococcus aureus*, was used to determine the antibacterial activity of each honey sample.

A sizable colony of *Staphylococcus aureus* was emulsified in 4 ml of normal saline, yielding approximately 1.0 x 10^6 per ml, and used to swab agar plates of diagnostic sensitivity test [(DST), Oxoid, England]. Four wells of 6 mm diameters each, were made on each plate and 50 µL of each honey dilution was placed into one well with a chipped tip pipette. Each honey sample was tested in triplicate. The plates were left at room temperature for 10 minutes prior to incubation until the honey had sipped into the agar, then incubated for 24 hours at 37°C. After incubation, the diameter of inhibition zones were measured in millimeters (mm) and the average recorded.

Gentamicin preparation. A vial of 1 g gentamicin was diluted to 8 µg/ml. This was divided into small aliquots and frozen at –70°C. Each time a test was performed, a fresh vial was thawed and used only once.

Mixing. A 2 ml sample of honey was mixed with equal volume of gentamicin (8 µg/ml) and vigorously vortexed to get a homogenous mixture of gentamicin (4 µg/ml) and honey (50% concentration). A sample of honey was diluted by equal amount of broth (1:2 dilution) and used along side the honey with gentamicin.

A test set was run with 3 tubes of: honey (50% concentration), gentamicin (4 µg/ml), and honey plus gentamicin (50% concentration, 4 µg/ml). These 3 samples were carried out in triplicates, so that a total of 9 tubes were set up. To each test tube, 20 µL of *Staphylococcus aureus* (10^6) was added and vortexed. Immediately after this, 20 µL were taken from each tube and mixed with 180 µL of saline (1:10 dilution). From each of the dilutions, 10 µL were plated on to the surface of blood agar and incubated for 24 hours at 37°C. This exercise of 1:10 diluting and sub-culturing was repeated for each tube, every 0.5 hours for 2 hours and then every 2 hours for up to 8 hours and then finally at 24 hours. These tests were run 5 times on different days. All growth on blood agar plates was counted and multiplied by 100 to arrive at numbers of organisms per ml in the final dilution of 1:10, then multiplied by 10 to give the number of organisms per ml surviving in the test runs at different periods of incubation. The triplicate runs for each experiment with honey, gentamicin or honey plus gentamicin were averaged. The results of the 5 different experiments for each substance were averaged again to give the final performance in killing ability.

Results. When all honey samples were tested for their activity, an arbitrary definition to grade the honey activities was used;10 samples with zones of inhibition in diameter of: >30 mm were rated as excellent, 21-29 mm as good, 10-20 mm as fair and <10 mm as poor. By this ranking, 13 (43%) of all honey tested had excellent activity against *Staphylococcus aureus* (**Table 1**). Ten samples (33%) showed good activity, 5 samples (17%) showed fair activity and 2 samples (7%) failed to show any detectable activity. One honey sample, OH26, from Al Batinah region (Al Kaborah) showed the highest level of anti *Staphylococcus aureus* activity. The antibacterial activity was evident up to 1 in 8 dilutions (12.5% concentration). This sample (OH26) was used for testing in combination with gentamicin. **Figure 1** demonstrates the zones of inhibition of the various dilutions of this honey sample.

Results of OH26, in combination with gentamicin are shown in **Table 2** and **Figure 2**. **Table 2** and **Figure 2** show the killing rates of *Staphylococcus aureus* by the 3 substances. The means of killed organisms at different times are given in percentages. Honey killed 38% of the organisms within half an hour, 79% in 6 hours and all organisms were killed at 24 hours. For gentamicin, the killing rates were 70% in half hour, 88% at one hour, 99.5% at 2 hours and 100% at 4 hours. The killing rates of honey combined with gentamicin, were 92% in half an hour, 93% in one hour, 95% in 2 hours and 100% killing was achieved thereafter. Gentamicin and honey killed 22% more than gentamicin alone within 30 minutes, indicating an enhancement activity of honey to gentamicin ($p<0.05$).

Discussion. The emergence of multiple antibiotic resistant organisms in a community is a potentially serious threat to public health. The emergence of antibiotic resistance has not yet prompted a radical revision of antibiotic utilization.[9] Instead it has prompted the development of additional antibiotics.[9] Where appropriate;

alternatives to antibiotics ought to be considered. There are already several non-antibiotic approaches to the treatment and prevention of infection, including the use of honey for wound infections.[2,9] Although many studies of antimicrobial activity of honey exist, this is the first study to address the antimicrobial effects of Omani honey in combination with an antibiotic. The anti *Staphylococcus aureus* activity of the Omani honey varies considerably with some samples possess excellent bacteria inhibitory activity, while others have good and some have fair or no activity at all. This study, confirms our previous observations that not all types of honey posses the same antibacterial activity.[8]

One Omani honey sample (OH26) possessed the highest antibacterial activity among all the samples tested, in terms of having greater inhibition of *Staphylococcus aureus* growth. It had excellent antibacterial activity at 50% concentration and good activity up to a concentration of 12.5%, whereas all other samples lost their activity at this concentration. We are now planning to further characterized OH26 using modern analytical techniques to define the most vital component of this honey sample. It is interesting to note that when a sample has excellent or good activity against *Staphylococcus aureus*, it is likely to have a fair to good activity against *Escherichia coli* and *Pseudomonas aeruginosa* (Gram negative organisms) as well,[8] indicating that the antibacterial activity of honey is of a broad spectrum.

The chemical composition of each honey sample is attributable to the nectar of each flower from where the bees produced honey. Honey is known to contain fatty acids, lipids, amylases, ascorbic acid, peroxidases and fructose,[10-12] which may contribute to its antimicrobial activity. The high osmolarity, low pH (3.6-3.7), peroxidase, glucose and fructose contents of honey and its possession of tetracycline derivatives or fatty acids may all play a role in honey antibacterial actions.[9-11] The relative importance of the mentioned factors may depend on the sensitivity of the bacterial species and the level of additional factors of any honey sample. Although honey is known to have high therapeutic actions on different bacteria including those that are highly resistant to antibiotics,[5-7] the exact mode of action of honey against many microorganisms is still not clear at present.

From our previous[8] and present study, some honey samples possess excellent antibacterial activities and these could be used topically for treating bacterial infections particularly in areas where there is a shortage of antibiotics. Chronic wounds such as diabetic wounds, pressure ulcers, leg ulcers are a common problem among older people and honey may provide an alternative method to the current time-consuming and costly practices of wound management in the nursing home.[13] However, it is urged that either large quantities or topical use are the effective ways of application. Since honey is used for treatment of infections, other medications are commonly given at the same time including antibiotics. It is well known that drugs, food staff and other consumables may interact negatively or positively with the antibiotics. The result of this interaction, between honey and antibiotics, to our knowledge, has not been researched before. Additionally, the honey killing rate of microorganisms is unknown. This study has demonstrated that honey starts its action rapidly within half an hour, but then slows down to achieve 80% killing in the next 6 hours, thereafter kills all the *Staphylococci* within 24 hours. While gentamicin is bactericidal to *Staphylococcus* achieving 70% killing within 30 minutes, a combination of gentamicin and honey kills >20% by that time. This additional effect is less than the 1000 fold difference expected in synergism and, therefore, we have designated this effect as enhancement. At least, it is absolutely clear that honey has no antagonistic effect to aminoglycosides. However, more work is required to find out its effect on other antimicrobial groups and other microorganisms, especially those that are resistant to antibiotics.

In addition to the antibacterial activity of honey, it has its nutritional values and would be an additional enhancer of immunity in aid to the treatment of bacterial infections.

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References

1. Molan PC. The antibacterial activity of honey. ***Journal of Bee World*** 1992; 73: 131-135.
2. Lusby PE, Coombes A, Wilkinson JM. Honey: a potent agent for wound healing? ***J Wound Ostomy Continence Nurs*** 2002; 29: 295-300.
3. Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zealand honeys. ***J Pharm Pharmacol*** 1991; 43: 817-822.
4. Effem SE. Clinical observations on the wound healing properties of honey. ***Br J Surg*** 1998; 75: 697-681.
5. Cooper R, Molan P. The use of honey as an antiseptic in managing Pseudomonas infection. ***J Wound Care*** 1999; 8: 161-164.
6. Nzeako BC, Hamdi J. Antimicrobial potential of honey on some microbial isolates. ***Sultan Qaboos University Journal of Medical Sciences*** 2000; 2: 75-79.
7. Karayil S, Deshpande SD, Koppikar GV. Effect of honey on multidrug resistant organisms and its synergistic action with three common antibiotics. ***J Postgrad Med*** 1998; 44: 93-96.

8. Al-Jabri AA, Nzeako B, Al Mahrooqi Z, Al Naqdy A, Nsanze H. In vitro antibacterial activity of Omani and African honey. ***Br J Biomed Sci*** 2003; 60: 1-4.
9. Carson CF, Riley TV. Non-antibiotic therapies for infectious diseases. ***Commun Dis Intell*** 2003; 27: S143-146.
10. Oka H, Ihai Y, Kawamura N, Una K, Yamada M, Harada K, Suzuki M. Improvement of chemical analysis of antibiotics: Simultaneous analysis of seven tetracyclines in honey. ***J Chromotogr*** 1987; 400: 253-261.
11. White JW, Subers MJ, Scheparto AI. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxidase and its origin in a honey glucose oxidase system. ***Biochim Biophys Acta*** 1963; 73: 57-70.
12. Wahdan HA. Causes of the antimicrobial activity of honey. ***Infection*** 1988; 26: 26-31.
13. Van der Weyden EA. The use of honey for the treatment of two patients with pressure ulcers. ***Br J Community Nurs*** 2003; 8: S14-20.