ORIGINAL ARTICLE

Antimicrobial effect of different types of honey on Staphylococcus aureus

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Abstract Honey exhibits antimicrobial activities against a wide range of bacteria in different milieu. This study aims to compare the effects of five types of honey (both imported and local Saudi honey) against Staphylococcus aureus. The five types of honey (Manuka Honey UMF +20, Manuka Honey UMF +16, Active +10 Manuka Honey, Sidr honey and Nigella sativa honey) were evaluated for their bactericidal/bacteriostatic activities against both methicillin resistant and sensitive S. aureus. The inhibitory effect of honey on bacterial growth was evident at concentrations of 20% and 10% (v/v). Manuka Honey showed the best results. Manuka Honey UMF +20 had a bactericidal effect on both methicillin resistant and sensitive S. aureus. However, Sidr and N. sativa honey exerted only a bacteriostatic effect. 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. Manuka Honey had the best bactericidal activity. Future experiments should be conducted to evaluate the effects of honey on bacterial resistance.

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1. Introduction

Honey is composed of approximately 82.4% total carbohydrates (38.5% fructose, 31.0% glucose and 12.9% from carbohydrates consisting of maltose, sucrose and other sugars)
The natural ingredients of honey show different activities against various microorganisms. Its activity is likely to be dependent on the grazing grounds and the weather conditions where the bees were raised, and on the natural structure of the blossom nectar (Abd-El Aal et al., 2007). Honey has an increasing effect on the levels of anti-oxidants, iron and rare elements in blood (Theunissen et al., 2001).

Abd-El Aal et al. (2007) showed that honey had a more pronounced inhibitory effect (85.7%) on Gram negative bacteria (Pseudomonas aeruginosaa, Enterobacter spp., Klebsiella) in comparison to commonly used antimicrobial agents. A 100% inhibition was observed in the case of Gram positive methicillin-resistant Staphylococcus aureus in comparison to the use of antibiotics alone. A synergistic effect was achieved upon the application of honey together with the antimicrobial agents in both Gram negative and positive bacteria. Al Somal et al. (1994) reported the inhibitory effect of Manuka Honey on Helicobacter pylori growth. In addition, it was documented that honey could completely heal severe injuries (Visavadia et al., 2008).

The use of honey as a drug for the treatment of disease dates back to 2100-2000 BC. For instance, pale honey was described by Aristotle (384-322 BC) as being "good for sore eyes and wounds" (Mandal and Mandal, 2011; Vallianou et al., 2014). The antimicrobial properties of honey have been well documented, and honey has been used from ancient times as a method of accelerating wound healing. Its potential to assist wound healing has been demonstrated repeatedly (Molan, 1999; Vallianou et al., 2014). A possible reason behind its activity relies on its ability to generate hydrogen peroxide by the bee-derived enzyme glucose oxidase (Saleh et al., 2011; Jing et al., 2014). Another possibility is the composition of honey, which has more than 181 constituents (Bogdanov and Martin, 2002; Gheldof et al., 2002; Mandal and Mandal, 2011; Vallianou et al., 2014).

Staphylococci bacteria are Gram-positive cocci (Ryan and Ray, 2004). The genus Staphylococcus is composed of 33 species (Bergey and Holt, 1994). Most staphylococci constitute the normal flora of the skin and mucous membranes (Midigan, 2005). Some are aerobic while others are anaerobic and can grow at high salt concentrations, reaching up to 10% (Murray et al., 2005). The most pathogenic species is S. aureus (Murray et al., 2005). Some coagulase-negative staphylococci (CNS) strains, causative agents of infection in immune-compromised individuals, developed resistance to antibiotics. These bacteria colonize devices that are implanted in the human body, such as nails, slides and industrial joints used in bones, heart valves and catheters of various types, as well as in peritoneal dialysis. It has been observed lately that there was an increase in the prevalence and incidence of methicillin resistant CNS and S. aureus, making it more challenging to treat such infections (Kloos and Bannerman, 1994). Coagulase-negative staphylococci are considered one of the most prevalent microorganisms that are involved in hospital-acquired infections (Tunney et al., 1996). Honey has been used to inhibit these bacteria as well as to prevent and treat skin and other infections (French et al., 2005).

The aim of the study was to evaluate the effect of different types of honey including two local honey on both methicillin sensitive and resistant S. aureus.

2. Methodology

2.1. Honey used

Five types of honey were used, namely Manuka Honey UMF +20 (SummerGlow Apiaries, New Zealand), Manuka Honey UMF +16 (SummerGlow Apiaries, New Zealand), Active +10 Manuka (Happy Valley Honey, New Zealand), Nigella sativa (Valley Honey, Kingdom of Saudi Arabia [KSA]), and Sidr (Valley Honey, KSA). The honey was kept in dark bottles away from sunlight. The age of the honey samples ranged from 6 to 10 months.

2.2. Physico-chemical properties

pH, moisture and sugar contents were determined according to the International Honey Commission (Bogdanov, 1984). Total phenolic content was measured as reported in the literature according to Velioglu et al. (1998) and Singleton et al. (1999).

2.3. Bacteria used

a. Methicillin sensitive S. aureus (MSSA): Ten strains of clinical isolates of MSSA were used. A control strain (ATCC MSSA) was also included (ATCC 29213, USA). The clinical isolates came from two different hospitals: King Abdulaziz Hospital, Jeddah, KSA and King Fahd Hospital, Jeddah, KSA.

b. Methicillin resistant S. aureus: Ten strains of clinical isolates of MRSA were used. This is in addition to one ATCC MRSA (ATCC 26112, USA). The clinical isolates came from two different hospitals King Abdulaziz Hospital, Jeddah, KSA and King Fahd Hospital, Jeddah, KSA.

3. Study design

Nutrient broth (NB) (Oxoid, U.K.) was used as a culture medium. Overnight cultures of both MSSA and MRSA were grown in NB. Bacterial strains were grown in the presence and absence (control) of different concentrations of honey. This was achieved by inoculating 0.1 ml of the overnight culture was inoculated into tubes containing 10 ml of NB and incubated overnight in the presence different concentrations of honey 0%, 10% (v/v), 20% (v/v) and 50% (v/v). Honey concentrations are expressed as percentage and weight at a density of 1.42 g/ml: 10% (v/v) (0.142 g/ml), 20% (v/v) (0.284 g/ml), and 50% (v/v) (0.701 g/ml).

a. Nutrient broth: bacterial counts were then done by preparing serial dilutions and colonies were counted on NB agar. The percent decline was determined in comparison to the control.

b. Agar: To evaluate the antimicrobial efficacy of honey using Mueller–Hinton Agar (HiMedia Laboratories, Mumbai, India), different concentrations of honey were added to molten warm agar (50-55 °C) to achieve the required concentration in the resulting Petri dishes which were used for the growth of the test bacteria. 0.1 ml of each one of the tested bacteria was
inoculated onto those plates at 37 °C and incubated for 24 h and then counted.

3.1. Bactericidal/bacteriostatic effects of honey

In order to evaluate the bacteriostatic/bactericidal activity of honey, 50% (v/v) of each one of following types of honey: Manuka Honey UMF +20, Sidr honey and *N. sativa*, were dispensed into sterile tubes. 0.1 ml of each of the test bacterial strains was inoculated into those tubes and incubated for 24 h at 37 °C. This concentration resulted in the complete inhibition of bacterial growth. In order to verify whether the honey has a bacteriostatic/bactericidal, 1 ml was added to 9 ml broth without honey and incubated for 24, 48 and 72 h. 0.1 ml of those cultures was placed on nutrient agar (free of honey) for 24 h to check for signs of bacterial growth. the honey type was considered as bacteriostatic if growth occurred and bactericidal when inhibition of growth persisted.

3.2. Statistical analysis

One-way ANOVA was used to investigate whether there was a significant difference among the various experiments. The *t*-test was used to compare variables between MSSA and MRSA. *P*-values < 0.05 were considered significant.

4. Results

4.1. Physico-chemical properties

Table 1 shows the physico-chemical properties of the three different types of honey used (average ± standard deviation). The average densities (g/ml) for the honeys were as follows: 1.47 ± 0.04 (Manuka), 1.433 ± 0.01 (*N. sativa*) and 1.47 ± 0.01 (Sidr) and the differences were not significant among the different types used (*P* > 0.05). The local Sidr honey was the least acidic with a mean pH value of 6.00 ± 0.02 and the differences were significant (*P* < 0.05). There was also a significant difference in the mean sugar contents per 100 g of honey with values of 82.00 ± 0.58% (Manuka), 81.14% ± 0.95% (Sidr) and highest 92.05 ± 0.84 (N. sativa). Furthermore, the mean percentage moisture contents per 100 g of honey were 17.40% ± 0.00% (Manuka), 14.60% ± 0.58 (Nigella stiva) and 14.30% ± 0.58 (Sidr). Manuka Honey had a high total phenol content of 103.99 ± 1.68 mg GAEs/kg compared to 81.30 ± 0.02 mg GAEs/kg for *Nigella sativa* and 96.00 ± 0.02 mg GAEs/kg for Sidr. Regarding the phenol content

| Parameters | Manuka | *Nigella sativa* | Sidr |
|------------|--------|-----------------|------|
| Density (g/mL) | 1.47 ± 0.04 | 1.433 ± 0.01 | 1.47 ± 0.01 |
| Moisture %/100 g | 17.40 ± 0.00 | 14.60 ± 0.58 | 14.30 ± 0.58 |
| pH | 4.30 ± 0.04 | 4.43 ± 0.06 | 6.0 ± 0.20 |
| Sugar content %/100 g | 82.00 ± 0.58 | 92.05 ± 0.84 | 81.14 ± 0.95 |
| Total phenol (mg GAEs/kg) | 103.99 ± 1.68 | 81.30 ± 0.02 | 96.00 ± 0.02 |

However, when a given parameter of a certain type of honey is indicated by the same letter, this means that there was no statistical significance (*P* > 0.05).

a,b,c The subscripts a, b, and c represent which honeys are significantly different from one another.

Table 2 Effect of different concentrations of honey on both methicillin sensitive and resistant *Staphylococcus aureus* using broth dilution method.

| Concentration | Bacteria | Mean MSSA (CFU/ml) | Mean MRSA (CFU/ml) |
|---------------|----------|--------------------|--------------------|
| 10% (v/v)     | Control  | 3.40 $\times 10^4$ | 5.50 $\times 10^6$ |
|               | Manuka +10 | 3.70 $\times 10^4$ | 5.50 $\times 10^6$ |
|               | Manuka +16 | 4.00 $\times 10^4$ | 5.05 $\times 10^6$ |
|               | Manuka +20 | 0.33 $\times 10^4$ | 0.50 $\times 10^5$ |
|               | *Nigella sativa* | 3.70 $\times 10^6$ | 5.50 $\times 10^5$ |
|               | Sidr     | 3.67 $\times 10^4$ | 1.00 $\times 10^5$ |
| 20% (v/v)     | Manuka +10 | 4.00 $\times 10^5$ | 5.00 $\times 10^6$ |
|               | Manuka +16 | 0.33 $\times 10^5$ | 5.00 $\times 10^6$ |
|               | Manuka +20 | 0.00 | 0.00 |
|               | *Nigella sativa* | 7.00 $\times 10^4$ | 5.50 $\times 10^4$ |
|               | Sidr     | 3.67 $\times 10^4$ | 1.00 $\times 10^5$ |
| 50% (v/v)     | Manuka +10 | 0.00 | 0.00 |
|               | Manuka +16 | 0.00 | 0.00 |
|               | Manuka +20 | 0.00 | 0.00 |
|               | *Nigella sativa* | 0.00 | 0.00 |
|               | Sidr     | 0.00 | 0.00 |

Abbreviations: MSSA: methicillin sensitive *Staphylococcus aureus*. MRSA: methicillin resistant *Staphylococcus aureus*.

* Differences between controls and the tested honeys were significant at the 0.05 level.
the difference was significant among the three types of honey \((P < 0.05)\) with Manuka having the highest phenol content of 103.99 ± 1.68.

4.2. Effect of honey on MSSA and MRSA in broth and agar

For all tested honey types, there was total inhibition of bacterial growth in both MSSA and MRSA at the highest concentration of 50\% \((v/v)\) (Table 2). At a concentration of 10\% \((v/v)\), all three tested Manuka honeys produced a significant decline in both MSSA and MRSA; \(N.\ satsiva\) and Sidr honey did not cause a significant decrease in bacterial growth (Table 2). A broader effect was evident when testing was done with honey at 20\% \((v/v)\) concentration. All types of honey caused a significant decline in bacterial growth for MSSA and MRSA. The same observation was noted when the agar method was used, except for MSSA with 20\% \((v/v)\) Sidr honey (Table 3). Statistical analysis using the \(t\)-test analysis showed that there was no significant difference in the averages of the effect of different types of honey on each of the MSSA and MRSA strains \((P = 0.480; \text{Table } 4)\).

4.3. Bacteriostatic/bactericidal efficacy of honey

Table 5 demonstrates the inhibitory action of Manuka, \(N.\ satisiva\), and Sidr honey on the tested bacterial strains.

Manuka Honey had a bactericidal effect, while the other two types of local honey possessed a bacteriostatic ability.

5. Discussion

Published data indicate that natural honey consists mainly of carbohydrates (about 82\%), water and other minor components. Those minor ingredients include: proteins, minerals, phytochemicals and antioxidants. It has been reported that those minor ingredients are the ones that are responsible for medical and biological activities of honey in the treatment of infections, burns, wounds and ulcers (Moumbe et al., 2013). The honey sugars are mostly fructose (38.2\%) and glucose (31.2\%), sucrose concentration ranges between (0.7\% and 1\%), disaccharides (approximately 9\%) some trisaccharides and higher saccharides (Aiken et al., 2012).

All the honey tested fell within the acceptable ranges concerning acceptable water contents between 13.66\% and 25.35\% (Moumbe et al., 2013). Such levels are low to allow yeast fermentation as well as bacterial growth (Boateng and Diunase, 2015). The high sugar content noted in Manuka and Sidr was comparable with \(N.\ satisiva\) having a significantly higher sugar content among the three honeys. In the case of acidity, Manuka and \(N.\ satisiva\) were comparable, while Sidr was the least acidic. The acidity is likely to contribute to the antibacterial potency of the honey (Boateng and Diunase, 2015). In accordance with what has been published earlier,

| Table 3 | Effect of different concentrations of honey on both methicillin sensitive and resistant \(Staphylococcus\ aureus\) using the agar dilution method. |
|----------|----------------------------------------------------------------------------------------------------------------------------------|
| Concentration | Bacteria | Mean MSSA (CFU/ml) | Mean MRSA (CFU/ml) |
| 10\% \((v/v)\) | Control | 2.03 × 10⁸ | 3.90 × 10⁸ |
| | Manuka + 10 | 3.75 × 10⁸* | 4.55 × 10⁸* |
| | Manuka + 16 | 4.19 × 10⁸* | 5.15 × 10⁸* |
| | Manuka + 20 | 2.10 × 10⁸* | 1.19 × 10⁸* |
| | \(Nigella\ sativa\) | 1.55 × 10⁸ | 3.90 × 10⁸ |
| | Sidr | 1.55 × 10⁸ | 3.90 × 10⁸ |
| 20\% \((v/v)\) | Manuka + 10 | 1.02 × 10⁸* | 1.00 × 10⁸* |
| | Manuka + 16 | 2.50 × 10⁷* | 0.00* |
| | Manuka + 20 | 1.00 × 10⁷* | 0.00* |
| | \(Nigella\ sativa\) | 7.27 × 10⁷* | 8.60 × 10⁷ |
| | Sidr | 9.97 × 10⁷ | 1.50 × 10⁸ |
| 50\% \((v/v)\) | Manuka + 10 | 0.00* | 0.00* |
| | Manuka + 16 | 0.00* | 0.00* |
| | Manuka + 20 | 0.00* | 0.00* |
| | \(Nigella\ sativa\) | 0.00* | 0.00* |
| | Sidr | 0.00* | 0.00* |

Abbreviations: MSSA: methicillin sensitive \(Staphylococcus\ aureus\). MRSA: methicillin resistant \(Staphylococcus\ aureus\).

* Differences between controls and the tested honeys were significant at the 0.05 level.

| Table 4 | Comparison effect of honey on methicillin sensitive and resistant \(Staphylococcus\ aureus\). |
|----------|----------------------------------------------------------------------------------------------------------------------------------|
| Number of samples | Mean (CFU/ml) | Std. error mean | \(P\)-value |
| MSSA | 198 | 7.2082 × 10⁶ | 1.62294 × 10⁶ | .480 |
| MRSA | 198 | 8.9577 × 10⁶ | 1.87161 × 10⁶ | |

Abbreviations: MSSA: methicillin sensitive \(Staphylococcus\ aureus\). MRSA: methicillin resistant \(Staphylococcus\ aureus\).
the data showed that Manuka Honey contained the highest amount of total phenolic compounds such as methyl syringate which provides this honey with its ability to scavenge potent superoxide free radicals and, thus, exerts its antibacterial activity (Alsarra, 2009; Muzzarelli et al., 2012).

Different types of honey possess different efficacies and mechanisms against the same type of bacteria, with Manuka Honey showing the best performance. This is in accordance with what has been reported by other authors who used honey as an agent to inhibit biofilm formation by *S. aureus* (Lu et al., 2014). Another study showed that at a concentration less than 10%, Manuka Honey was needed for the inhibition of all the 58 strains of Gram-positive MSSA and 18 strains of MRSA isolated from wounds (Cooper et al., 1999, 2002). The antibacterial effects of honey are not only due to its osmolarity, but also due to other important factors that are present in the composition of honey (Carnwath et al., 2014; Cooper et al., 2002). Such factors 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 (Allen et al., 1991; Molan, 1999; Sherlock et al., 2010; Mandal and Mandal, 2011; Al-Waili et al., 2013; Jing et al., 2014; Vallianou et al., 2014). Consequently, the tested honey samples were kept in dark bottles away from sunlight and in a refrigerator (Irish et al., 2011). The age of the honey samples ranged from 6 to 10 months.

Moreover, honey contains several vitamins and minerals (Ajibola et al., 2012; Vallianou et al., 2014). It also contains amino acids, antibiotic-rich proteins, phenol antioxidants, and other biologically active compounds (Beretta et al., 2010; Ramirez-Arriaga et al., 2011; Wang and Li, 2011; Vallianou et al., 2014). Some types of honey also contain kynurenic acid, which may contribute to its antimicrobial properties (Beretta et al., 2007; Vallianou et al., 2014).

In this study, the type of honey and the concentration affected its bactericidal and bacteriostatic activities. This is in line with the report of other authors who found that honey was effective against antibiotic-resistant bacteria that colonize burn wounds, such as MRSA, vancomycin-resistant *Enterococcus* spp. (VRE) and multiple-resistant Gram-negative rods, including *P. aeruginosa*, *Acinetobacter* spp. and members of the Enterobacteriaceae family (Hussein et al., 2012; Al-Waili et al., 2012; Vallianou et al., 2014). Studies have shown that Manuka Honey was effective against bacterial biofilms of Group A *Streptococcus pyogenes*, *Streptococcus mutans*, *Propionibacterium acnes*, *Enterobacter cloacae* and *S. aureus* (Alandejani et al., 2008; Stephens et al., 2010; Maddocks et al., 2013; Majtan et al., 2014). Manuka Honey is produced from the nectar of the Manuka bush (*Leptospermum scoparium*), which is indigenous to New Zealand and Australia. Manuka exceptionally contains high concentrations of the anti-bacterial compound methylglyoxal, which may be the reason behind its high bactericidal activity. This non-peroxide antibacterial activity due to the presence of methylglyoxal is called the unique Manuka factor (UMF) (Muzzarelli et al., 2012). However, the exact compound(s) that contribute to its activity have not yet been fully elucidated (Molan, 1992; Adams et al., 2008; Irish et al., 2011; Vallianou et al., 2014).

Manuka Honey’s antibacterial activity is not linked to hydrogen peroxide (H₂O₂) (Irish et al., 2011). Therefore, the effect of this type of honey on microbes may be due to its low pH or high sugar content, or as mentioned previously. It was noted by Irish et al. (2011) that the efficacy of H₂O₂ based honey was compromised by temperature, while non-H₂O₂ based honey were not affected. This explains why the different types of honey may differ in their antimicrobial potential, depending on the time, storage, composition and source of nectar on which the reared bees were fed (Irish et al., 2011; Mandal and Mandal, 2011). Jenkins et al. (2011) reported that Manuka Honey inhibited the growth MRSA by preventing cell division and potentiating oxacillin inhibition on MRSA.

Our results were in agreement with other published studies, showing that at a concentration of 50% (v/v) in a NB medium, Black Angenaz honey and Active Manuka Honey (AMH), Unique Manuka Factor (UMF) honey had an inhibitory effect against the fungus *Candida albicans*, *P. aeruginosa*, vancomycin resistant enterococci and MSSA (Al-Boraikan, 2006). The results indicated that UMF honey exerted a bactericidal effect on all microorganisms tested while AMH honey and Angenaz Black honey had only an inhibitory effect against all the tested bacteria, except for MSSA. Mohapatra et al. (2011) also reported that honey was effective against both Gram-positive (*S. aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Enterococcus faecalis*, and *Micrococcus luteus*) and Gram-negative bacteria (*Escherichia coli*, *P. aeruginosa*, and *Salmonella typhi*); this effect was either bacteriostatic or bactericidal. This is similar to the results obtained in this study, where sensitivity dependent on the type of honey and the concentration at which it was applied. It was reported that the inhibitory potential of Manuka Honey on MRSA appeared only at concentrations above 12.5% (v/v). In contrast, Ulmo 90 honey was bactericidal on MRSA at concentrations of 6.3% (v/v) and 3.1% (v/v). In the same study, Manuka Honey (particularly UMF + 20) showed a high inhibitory effect on bacteria (Sherlock et al., 2010).

Al-Haj et al. (2009) performed a study in which they tested Malaysian honey on both MSSA and MRSA. They concluded that honey completely inhibited bacterial growth. In another study, Merckoll et al. (2009) showed that biofilms were abolished by the biocidal substances present in Norwegian honey, and the honey was good for wound care.

Up till now, there has been no report of bacterial resistance to honey. This is likely due to the complex composition of honey, which causes the individual components to act either individually or in synergy to prevent resistance (Cooper et al., 2010).

In conclusion, 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. Manuka Honey fortified with 20 UMF had highest bactericidal activity
and proved to reduce the pattern of resistance of \textit{S. aureus} compared to commonly used antibiotics. For the Islamic world, the potential use of honey has been documented in the Quran as a good source of treatment against many diseases. Future studies should focus on the use of local Saudi honey in combating antimicrobial resistance and as complementary to other antimicrobial dressings, since it is readily available in most parts of Middle East and relatively cheap, compared to mainstream antibiotics, in addition to its use in the treatment of wounds, ulcers and cuts, since it has antibacterial activity and enhancing healing. Also, Future studies should pinpoint the functional components in a wide variety of local Saudi honeys and test their biological activities.

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