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ORIGINAL ARTICLE

Antimicrobial activities of Saudi honey against *Pseudomonas aeruginosa*

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Abstract Five types of imported and local honey were screened for both their bacteriocidal/bacteriostatic activities against both Imipenem resistant and sensitive *Pseudomonas aeruginosa* in both Brain Heart infusion broth and Mueller–Hinton agar. The results indicated that the effect was concentration and type of honey dependant. All types of honey tested exerted a full inhibition of bacterial growth at the highest concentration tested of 50% at 24 h of contact. The inhibitory effect of honey on bacterial growth was clear with concentrations of 20% and 10% and this effect was most evident in the case of Manuka honey as compared to *Nigella sativa* honey and Seder honey. Manuka honey UMF +20 showed a bacteriocidal activity on both Imipenem resistant and sensitive *P. aeruginosa*, while Seder honey and *N. sativa* honey exerted only a bacteriostatic effect. Manuka honey UMF +10 showed most effect on antimicrobial resistance. Manuka honey UMF +10 had an effect on modulation of Imipenem resistant *P. aeruginosa*. Conclusion: The results indicated that various types of honey affected the test organisms differently. Modulation of antimicrobial resistance was seen in the case Manuka honey UMF +10.

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1. Introduction

Honey is composed of approximately 82.4% total carbohydrates which include mainly 38.5% fructose and 31% glucose. Other sugars like maltose, sucrose and others constitute 12.9% of its composition (Khan et al., 2007). The efficacy of honey against different types of microbes is dependent on many

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factors such as: the type and natural structure of the nectar as well as the weather conditions where the bees were reared (Abd-ElAal et al., 2007). Bacterial resistance is less likely to develop as a result of treatment of bacteria with honey. This is because of the composition of honey which contains a number of different components (Carnwath et al., 2014). Gram negative organisms such as *Pseudomonas aeruginosa* have been a major problem in hospital acquired infections and cause most severe wound and burn infections (Kronda et al., 2013; Roberts et al., 2012). *P. aeruginosa* has become multidrug resistant due to its potential to acquire new antimicrobial resistance (Roberts et al., 2012; Camplin and Maddocks, 2014). Its activity is enhanced by its ability to form biofilms and become resistant and evade the activities of the therapeutic agents (Campeau and Patel, 2014). Manuka honey has shown a synergistic effect when used together with vancomycin against *Staphylococcus aureus* biofilms, while only an additive effect was noted when used for treatment of *P. aeruginosa* biofilms (Campeau and Patel, 2014). The objective of this study was to evaluate the antimicrobial potential of various types of Saudi and imported honey against *P. aeruginosa*.

2. Methodology

2.1. Honey used

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 from Kingdom of Saudi Arabia (KSA), Seder (Valley honey from KSA).

2.2. Test bacteria

a. *P. aeruginosa* (Imipenem-sensitive): Ten strains of clinical isolates were used. In addition to one *P. aeruginosa* (ATCC 27853, USA) which served as control. The clinical isolates came from two different hospitals (King Abdulaziz and Oncology Center Hospital, Jeddah and King Fahd Hospital, Jeddah). They were isolated from the following infected sites: Wound swabs (3 isolates), Sputum (2 isolates), Blood (2 isolates), and one isolate each of Cerebral seminal fluid, Pus, and Ear.
b. *P. aeruginosa* (Imipenem-resistant): Ten strains of clinical isolates were used which came from (King Abdulaziz Hospital and Oncology Center, Jeddah) were isolated from the following infected sites: Sputum (5 isolates), Blood (2 isolates), and one isolate of each of the listed: Urine, Wound, and Tracheal aspirate.

2.3. Study design

2.3.1. Bacterial culture and application of honey

A. Broth: Cultures of either Imipenem resistant and sensitive *P. aeruginosa* were grown in Nutrient Broth (NB) overnight (Oxoid, U.K.) in the presence and absence of various honey concentrations. A 0.1 ml of the overnight culture (10^7 CFU/ml) was added to a tube containing 10 ml of sterile NB in the presence of different concentrations of honey (0, 10%, 20% and 50%) and incubated for 24 h at 37 °C. The cultures were serially diluted and counted on nutrient broth agar. The percent decline was determined in terms of the control. The data shown in the table represent the mean of different experiments done.
B. Agar: The Mueller–Hinton Agar was used (Hi-Media Laboratories, Mumbai, India). Different concentrations of Honey were impregnated and diluted with molten agar warm agar (50–55 °C). 0.1 ml (10^7 CFU/ml) of each one of the tested bacteria were inoculated onto those plates overnight at 37 °C and counted the next day.
C. Bacteriocidal/bacteriostatic: In order to evaluate the bacteriocidal/bacteriostatic effect of honey, three types of honey Manuka Honey UMF +20; Seder honey and *N. sativa* honey were tested at the highest concentration of 50% which achieved complete inhibition of bacterial growth at 24 h of incubation. This was done by inoculating 1 ml of the latter cultures into 10 ml broth and incubated for 24, 48 and 72 h to look for signs of growth. Samples were taken and checked for bacterial growth by streak plating.
D. Effect of honey on modulation of antimicrobial resistance: The effect of honey on the imipenem resistant *P. aeruginosa* was evaluated using different types of honey applied at 10% concentration using the Kirby–Bauer method of susceptibility testing (Harakeh et al., 2009).

2.4. Statistical analysis

One way ANOVA was used to investigate whether there was a significant difference among various experiments used. P values $\leqslant$ to 0.05 were considered significant.

3. Results

Using broth as a support medium, there was a complete inhibition of bacterial growth at 50% concentration of honey against both imipenem sensitive and resistant *P. aeruginosa*. At 20% concentration of honey, Manuka +20 produced a total reduction in bacteria for both organisms with significant reduction in bacterial counts with other types of honey. Also, there was complete cessation of bacterial growth in the case of Manuka +10 against imipenem sensitive *P. aeruginosa* at 10% concentration (Table 1).

Table 2 shows the effect of different types of honey on growth of imipenem sensitive and resistant *P. aeruginosa*, in agar. At 50% concentration all the types of honey resulted in total inhibition of bacterial growth, the same was true in case of 20% concentration except for Seder honey which caused only a significant decline in bacterial growth. In the case of 10% honey concentration, total inhibition of bacterial growth of both organisms was seen in Manuka +16 and +20. However, other types of honey caused a significant decline in bacterial numbers except for Seder honey.

Table 1 Effect of honey on both Imipenem resistant and sensitive *Pseudomonas aeruginosa* in broth.

Concentration		Bacteria	
		Mean Imipenem sensitive *P. aeruginosa*	Mean Imipenem resistant *P. aeruginosa*
10%	Control	3.4×10^{7}	1×10^{8}
	Manuka +10	2.3×10^{3}*	3×10^{2}*
	Manuka +16	3.3×10^{2}*	1×10^{2}*
	Manuka +20	0*	1×10*
	Nigella sativa	3.7×10^{4}*	1×10^{4}*
	Seder	3.367×10^{7}	1×10^{8}
20%	Manuka +10	6.7×10*	0*
	Manuka +16	3.7×10*	1.0×10*
	Manuka +20	0*	0*
	Nigella sativa	3.3×10^{2}*	5×10*
	Seder	3.3×10^{3}*	1.0×10^{2}*
50%	Manuka +10	0*	0*
	Manuka +16	0*	0*
	Manuka +20	0*	0*
	Nigella sativa	0*	0*
	Seder	0*	0*

* Significant at $P < 0.05$ level.

As shown in Table 3 all Manuka types of honey showed bactericidal effects against the organisms tested, where as the other types showed only a bacteriostatic activity.

Table 4 shows the effect of honey on modulation Antimicrobial Resistance of imipenem resistance *P. aeruginosa*. The results indicated that the effect was dependent on the type of honey used and Manuka +10 was the only one effective.

4. Discussion

The medicinal effects of honey date back to the days of Aristotle (384–322 BC) for the treatment of sore eyes and wound infections (Mandal and Mandal, 2011; Vallianou et al., 2014). The antimicrobial characteristics of honey have been established for a long time especially for wound healing (Cooper and Molan, 1999; Vallianou et al., 2014). Its activity may be due to its complex composition and its ability to generate hydrogen peroxide by the bee-derived enzyme glucose oxidase (Stephens et al., 2009; Lu et al., 2014; Bogdanov et al., 2008; Gheldof et al., 2002; Vallianou et al., 2014).

The results of this study showed that Manuka +20 has a strong antibacterial activity against both imipenem sensitive and resistant *P. aeruginosa*. No growth was noticed even at 10% concentration on Manuka +20. These results were in accordance with a study conducted by Henriques et al. (2011) about the effect of Manuka honey on the form of the bacterial cell *P. aeruginosa* ATCC 27853 by measuring the Minimum Inhibitory Concentration (MIC), Minimum Bacteriocidal Concentration (MBC), Minimum bacterial concentration in a manner of microtiter plate which was observed and examined by Scanning and transmission electron microscopy, the result was that the degree of MIC, MBC in honey Al-Manuka against *P. aeruginosa* was 9.5% (w/v) and 12% (w/v) respectively. This summarizes that the Al-Manuka honey has a strong effect on the *P. aeruginosa* bacteria.

At concentrations 50% and 20% all five types of honey had an effect on both strains of *P. aeruginosa*. This result is in agreement with the study conducted by Wilkinson and Cavanagh (2005), using 13 kinds of honey and their effect on *P. aeruginosa*, they concluded that all types of honey studied had a killing effect on the tested bacteria.

Hegazi (2011) reported using ATCC strains that inhibition of bacterial growth was noted when studying the effects of 8 types of honey: Acacia honey, Citrus honey, Clover honey, Coriander honey, Cotton honey, Palm honey, Sesame honey and a sample of Saudi Seder honey, on 6 types of Gram positive and negative bacteria, including: *Klebsiella pneumonia*, *P. aeruginosa* and *Escherichia coli*. Mohapatra et al. (2011) showed that honey has an antibacterial effect against both Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Enterococcus faecalis*, and *Micrococcus*

Table 2 Effect of honey on both imipenem resistant and sensitive *Pseudomonas* in agar.

Concentration		Bacteria	
		Mean Imipenem sensitive *P. aeruginosa*	Mean Imipenem resistant *P. aeruginosa*
10%	Control	2.23×10^{8}	2.63×10^{8}
	Manuka +10	9.32×10^{2}*	1×10^{3}*
	Manuka +16	0*	0*
	Manuka +20	0*	0*
	Nigella sativa	6.755×10^{4}*	7.02×10^{4}*
	Seder	2.395×10^{7}	3.48×10^{7}
20%	Manuka +10	0*	0*
	Manuka +16	0*	0*
	Manuka +20	0*	0*
	Nigella sativa	0*	0*
	Seder	2.78×10^{4}*	2.85×10^{4}*
50%	Manuka +10	0*	0*
	Manuka +16	0*	0*
	Manuka +20	0*	0*
	Nigella sativa	0*	0*
	Seder	0*	0*

* Significant at $P < 0.05$ level.

Table 3 Bacteriostatic and bactericidal effect of honey on Imipenem resistant and sensitive *Pseudomonas aeruginosa*.

Bacterial strains	Manuka +20	*Nigella sativa*	Seder
Pseudomonas aeruginosa ATCC 27853	–	+	+
Pseudomonas aeruginosa (Imipenem-sensitive)	–	+	+
Pseudomonas aeruginosa (Imipenem-sensitive)	–	+	+

(–) Bactericidal effect; (+) Bactericidal effect.

Table 4 Effect of 10% of honey on Imipenem resistant *Pseudomonas aeruginosa*.

Bacterial strains	Seder honey	*Nigella sativa*	Manuka +10 (cm)
1	R	R	1.2
2	R	R	1.1
3	R	R	1.2
4	R	R	1.5
5	R	R	1.1
6	R	R	1.5
7	R	R	1.4
8	R	R	1

luteus) as well as anti Gram negative bacteria (*E. coli*, *P. aeruginosa*, and *Salmonella typhi*). This effect was either bacteriostatic or bactericidal depending on the type of honey tested. In another study, it was reported that antibacterial effect exhibited by honey was related to the levels of hydrogen peroxide present in the honey (Irish et al., 2011; Alnaimat et al., 2012). In addition, researchers at the Waikato Honey Research Unit (2012) in New Zealand attributed the antibacterial effect of honey was PH related which ranged from 3 to 4.5. At those PH levels, most bacteria grew best at nearly neutral PH ranging from 7 to 7.4. However, bacteria have been able to withstand the effects of honey by forming biofilms (Lu et al., 2013, 2014).

The mode of action for Manuka honey was reported to be due to extensive cell lysis after exposure to inhibitory concentrations of the honey (Roberts et al., 2012, 2015). The effect of honey on the modulation of bacterial resistance is very promising and may be related to the complex composition of honey used which contains a combination of components that may act in a synergistic manner to compromise the resistance. Published work on the effect of honey on the development of bacterial resistance indicated that it may be very low because of the variability in the composition among various types of honey which depends on the: (1) types of nectar that the bees fed, (2) the related weather conditions, (3) storage time and (4) conditions of preservation (Sherlock et al., 2010; Al-Waili and Boni, 2003).

Based on the above, it would be concluded that the antibacterial effect of different types of honey is type and concentration dependent. Therefore, using honey for treatment of infections may be worth perusing. Also, the possibility of mixing it with other commercially available antibiotics or other naturally derived compounds, proven antimicrobial potential, will be something to investigate further.

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