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Inhibitory Effect of Honey against Some Pathogenic Bacterial Species Isolated from Clinical Specimens

Hanadi Abdulqader Jasim[1], Basil Abdulzahra Abbas[2*], Hassan Alaa Farid[1], Mohammed Basim[1]

[1]*Department of Microbiology, College of Medicine, University of Basrah*

[2]*Department of Microbiology and Parasitology, College of Veterinary Medicine, University of Basrah*

ABSTRACT

There is an urgent need for alternative antimicrobial policies which led to a re-evaluation of therapeutic use of older treatments such as the honey. Honey is a product that obtained and collected naturally from flowers by bees. This study is aimed to evaluate the in vitro antibacterial effect of natural local Iraqi honey against different pathogenic bacterial isolates, and compare this effect to the activity of Amoxicillin and Ciprofloxacin. Experimental study carried out in Basra Medical College/Department of Microbiology from Nov. 2014 - Feb. 2015 in which two types of natural honey (Cedar and Rabia'), from 18 different sources were used to implicate the in Vitro antibacterial activity against 6 pathogenic bacterial isolates (Staphylococcus aureus, Staphylococcus epidermidis, Methicillin Resistant Staphylococcus aureus (MRSA), Proteus spp., Klebsiella spp., and Pseudomonas aeruginosa) and the result is compared to the activity of two antibiotics. 84 % of the isolates show positive result and the average zone of inhibition was 13.5 mm for S. aureus, 9.77 mm for S. epidermidis, 9.2 mm for Proteus spp. and 7.94 mm for Klebsiella spp., MRSA and Pseudomonas aeruginosa revealed surprising susceptibility for different types of honey (14.4 mm for MRSA and 10.6 mm for Pseudomonas aeruginosa). In conclusion, the bacterial isolates from clinical specimens show obvious and attractive susceptibility against different local honey types.

Key words Honey, Antimicrobial, *Staphylococcus aureus*, *Pseudomonas*, *Klebsiella spp*

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Corresponding author: Basil Abdulzahra Abbas
e-mail ✉ :basil.abbas@uobasrah.edu.iq
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INTRODUCTION

Globally the effectiveness of antimicrobial agents is decreased as the resistant pathogens develop. This resistance to antimicrobials becomes a serious threat to public health [1,2]. Therefore, there is an urgent need for alternative antimicrobial policies which led to a re-evaluation of therapeutic use of older treatments, such as plants and products of plant origin, such as honey [3- 5]. Honey is a product obtained naturally and collected from flowers by bees. It has many features, including those that are being followed supplementary food and diet, and it can be used in makeup products [6]. Recently, honey, which is an old biological therapy, has been re-prescribed by medical profession especially when classical modern drugs fail [7]. As long as 2000 years before bacteria was discovered to be the cause of infections, honey was used to treat infected wounds serving to treat ulcers [8]. Honey imparts pharmaceutical properties since it has antibacterial and antioxidant activities. So, it has been in use since old times as a drug in many types of treatments [9]. Although the honey activity against microorganism was reported firstly in 1892 [10], but its medicinal use has been continued till present time [11]. Principally the antimicrobial activity of honey is mainly due to its, high osmolality, release of hydrogen peroxide, acidic pH and non-peroxide factors that derived from plant [12-14]. Besides that, decreased water activity of honey is essential and effective in inhibition of bacterial growth [15]. The mild acidity of honey, ranging between 3.2 and 4.5. Gluconic acid is formed in honey when bees secrete the enzyme glucose oxidase, which catalysis the oxidation of glucose to gluconic acid. Honey acidity is inhibitory to many bacteria and could be enough to employ the inhibitory effect [16]. A major antibacterial agent in honey is hydrogen peroxide which appears with different concentrations in various honey types. Besides that, some types of honey composed of many natural antibacterial components [6]. Some researchers believe that hydrogen peroxide is the principal antibacterial agent [17,18]. Furthermore, honey can heal the infection by another ways, including: strengthening the immune system, the presence of anti-inflammatory and antioxidant activities, and by stimulating cell growth [19].

The purpose of this study is to estimate in vitro antibacterial action of 18 samples of two Iraqi honey types (Cedar and Al-Rabia') against six different pathogenic

bacterial isolates: *Staphylococcus aureus*, *Staphylococcus epidermidis*, Methicillin Resistant *Staphylococcus aureus* (MRSA) *Pseudomonas aeruginosa*, *Proteus spp*. and *Klebsiella spp*. and compare it to the activity of Amoxicillin and Ciprofloxacin.

MATERIALS AND METHODS

In vitro experimental study carried out in Al - Basra Medical College / Department of Microbiology from Nov. 2014 – Feb. 2015.

Bacterial isolates: Six types of bacterial isolates were identified from different clinical specimens. These bacterial species had been collected, cultured and diagnosed by using different bacteriological & biochemical methods [17]. Both Gram +ve and Gram –ve bacteria were involved.

Honey specimens: Different types of local honey were collected (n=18 samples) from different geographical area in Basra, and two other cities (*Karballa*a and *Erbil*). Those involved two major types of honey Cedar and Al-Rabia'. Samples were collected with sterile containers from different sources in aseptic conditions and transferred to the laboratory.

Antimicrobial activity procedure: The effect of 18 samples of local honey were examined against different bacterial species. Agar diffusion method (Kirby–Baure procedure) was used to estimate the activity in vitro. Bacterial isolates were sub cultured on nutrient agar plates (24 hours), those bacterial species were spread on Muller-Hinton agar which was used for determine the activity. Pores were punched out on the surface of Muller Hinton plates by using cork–pourer (0.4 mm). The inoculum was standardized by McFarland (0.5) for each tested bacterium [17]. Then by using sterile pipettes 0.1ml of each honey sample were transferred to put inside the pores. Plates were incubated at 37oC for 24 hours. Two types of antibiotic discs were tested for comparison with the effect of honey, these types include: amoxicillin (20 µg) and ciprofloxacin (10 µg). Antibiotic discs put on the surface of each plate. Diameter of zone of inhibition has been recorded and compared with that of antibiotic discs.

Statistical analysis: SPSS (Statistical Package for Social Sciences -Version 20) computer file was used for statistical analysis.

RESULTS

This study was conducted to investigate in vitro activity of different types of natural local Iraqi honey which have been collected from different places as shown in Table 1 against some clinical isolates.

Table 1: Geographical sources of different types of honey.

Location	Type	No. of sample
Basra / Abo Alkhasieb	Cedar	1,2,3,4,6,10,12
Basra / Abo Alkhasieb	Al-Rabia'	5,7,14
Erbil/ Shaqlawa – Aljabal	Cedar	8
Basra / Souq Albasra	Al-Rabia'	9
Basra / Faculity of Agriculture	Al-Rabia'	11
Basra / Alhartha	Cedar	13
Basra / Alzubair	Cedar	15, 16
Basra / Shatt Alarab – Altanuma	Cedar	17
Karbalaa / Aljadwal Algharbi	Al-Rabia'	18

Two major honey types had been collected (Cedar and Al-Rabia'). Where Cedar means: monofloral honey which comes from a single flower source. While Al-Rabia' means: mutltifloral honey which is derived from the nectar of more than one flower source. Those types of honey were tested against various bacterial species that isolated from clinical specimens (Table 2). Out of the different bacterial isolates, both Gram positive and Gram-negative bacteria had been identified which include: *Staphylococcus aureus, Staphylococcus epidermidis*, MRSA, *Proteus spp., Klebsiella spp*., and *Pseudomonas aeruginosa*.

Table 2: Types & clinical importance of selected clinical isolations.

Sorce of isolation	Clinical importance	Type of pathogen
Wound swab	Community acquired &Nosocomial infection , Skin infections	Staph. aureus
Nasal passage	Nosocomial infections , pneumonia , surgical site infections, sptecemia	MRSA
Wound swab	Normal flora	*Staph. epidermidis*

Burn swab	Wound infection , Diabetic foot ulcer, Urinary infections	*Pseudomonas aeruginosa*
Sputum	Pneumonia, Urinary Tract Infection , Upper Respiratory Tract Infection, Wound Infection, Meningitis, Septicemia	*Klebsiella spicies*
Urine sample (UTI)	Septicemia, urinary infections, Woundinfections	*Proteus species*

Antibacterial activity of Honey against both Staph aureus and *Staph epidermidis* (Gram +ve bacteria) has been revealed various potency (Table 3). The average of diameter of inhibition zone 13.5 against Staph aureus and 9.77 against Staph epidermidis. This antibacterial effect was compared with two types of antibiotics that revealed less activity against Staph aureus than with *Staph epidermidis*.

Table 3: Antibacterial activity of different types of honey (diameter mm) against Gram +Ve bacteria, compared to amoxicillin and ciprofloxacin.

Honey source	S. aureus (mm)	S. epidermidis (mm)	Honey source	S. aureus (mm)	S. epidermidis (mm)
1	20	10	10	15	14
2	-	10	11	16	18
3	11	8	12	15	9
4	10	2	13	10	20
5	20	-	14	10	12
6	12	-	15	12	8
7	17	-	16	17	15
8	20	20	17	18	17
9	-	8	18	20	5
Average diameter for honey				13.5	9.77
Average diameter for Amoxicillin				8	25
Average diameter for Ciprofloxacin				Resistant	15

The activity of honey against Gram –ve bacteria (*Pseudomonas*, *Proteus and Klebsiella*) was appeared with the average of diameter of inhibition zone (10.6, 9.2 and 7.94 mm) respectively (Table 4). Beside that the clinical isolates of *Pseudomonas spp*. had been shown the resistance to both Amoxicillin and Ciprofloxacin while appeared sensitivity against different types of honey except four types as shown in Table 4.

The results show that there is an obvious activity of honey against selected bacterial species which include both Gram +ve and Gram –ve (Table 3 & 4) and this activity is slightly higher for Gram +ve in compare with Gram -ve but this is not statistically significant (Table 6). All types of examined honey showed activity against bacterial species with different diameter zone of inhibition ranging from 2–22 mm in diameter (Tables 3 and 4).

Table 4: Antibacterial activity (diameter mm) of different types of honey against gram - ve bacteria, in compare to amoxicillin & ciprofloxacin.

Honey sample	*Pseudomonas* diameter of inhibition (mm)	*Proteus* diameter of inhibition (mm)	*Klebsiell*a diameter of inhibition (mm)
1	15	10	10
2	15	10	8
3	14	8	3
4	12	15	8
5	20	12	10
6	17	-	-
7	15	-	-
8	-	-	-

9	-	11	11
10	-	10	8
11	-	8	12
12	10	12	12
13	9	12	10
14	10	11	12
15	11	10	-
16	10	10	9
17	22	15	18
18	12	12	12
Average diameter	10.6	9.2	7.94
Amoxicillin	Resistant	22	23
Ciprofloxacin	Resistant	15	19

The antibacterial activity of examined honey types against MRSA was studied (Table 5). The results showed significant inhibition zone compared with that measured against antibiotics (Amoxicillin and Ciprofloxacin).

Table 5: Antibacterial activity (diameter, mm) of different types of honey against mrsa, in compare to amoxicillin & ciprofloxacin.

Honey sample	MRSA diameter of inhibition (mm)
1	18
2	10
3	12
4	10
5	20
6	15
7	17
8	20
9	-
10	16
11	16
12	17
13	11
14	14
15	12
16	18
17	18
18	16
Average diameter	14.4
Amoxicillin	Resistant
Ciprofloxacin	Resistant

Both of MRSA and *Pseudomonas aeruginosa* that have been reported as resistant pathogens for many antibiotics, were revealed susceptibility for different types of honey. Both MRSA and *Pseudomonas aeruginosa* revealed resistance to the antibiotics and show that there is no effectiveness from Amoxicillin & Ciprofloxacin (Tables 4 and 5).

Totally the effectiveness of different local honey against Gram +ve and Gram –ve bacteria seem to be similar without any significant differences (P value = 0.279) (Table 6). The Cedar honey type appeared more effective on the bacterial growth than Al-Rabia' type (P value=0.852). (Table 7).

Table 6: The effectiveness of honey on gram +ve & gram -ve bacteria.

	Effectiveness		Total
	Effective	Not effective	
Gram +Ve	48	6	54
	88.80%	11.20%	100%
Gram -Ve	44	10	54
	81.48%	18.52%	100%
Total	89	19	108
X2 = 1.174	df = 1	P value = 0.279	

Table 7: The effectiveness of various types of honey.

		Effectiveness		
Honey Type		Effective	Not effective	Total
	Cedar	61	11	72
		84.70%	15.30%	100%
	Al-Rabia	30	6	36
		83.30%	16.70%	100%
Total		91	17	108
	X2 = 0.035	df = 1	P value = 0.852	

DISCUSSION

Recently, the effectiveness of the antimicrobial remedy is decreased with arising of microbial resistance. The bacterial resistance occurs as a serious problem and threat for public health. For such reason researches had reported the antimicrobial activity of different biological materials such as honey.

The result shows in vitro activity of different types of natural local Iraqi honey (Table-1) which was collected from local areas against different pathogenic bacterial species that isolated from clinical cases. The inhibitory effect of examined honey (Cedar & Rabia) was measured against *Staphylococcus aureus, Staphylococcus epidermidis* & Gram – Ve: *pseudomonas spp*., *Proteus spp. & Klebsiella spp*. (Tables 1 and 2). Statistically there is no difference in the antimicrobial effectiveness against bacterial species by these two honey types.

This property of honey could be related to the fact that honey contains multiple antibacterial substances, such as high osmolarity, acidic pH, hydrogen peroxide and plant-derived flavonoids and phenolic substances. The pathogenic bacteria have been affected by these substances synergistically on multiple targets [20-22].

All types of examined honey showed activity against bacterial species with different diameter zone of inhibition ranging from 2–22 mm in diameter (Tables 3 and 4). The result was in agreement with those studies of Deb Mandal et al. [7]. The best to our knowledge that the more potent and more serious isolates in this study (MRSA & *pseudomonas aeruginosa*) are more susceptible to examined honey. That might be belong to the inhibitory effect of honey which results from more than one factor [13]. Different honey types revealed variable potency that resulted from different wild flowers grown in South Africa against S. aureus which showed antibacterial activity due to the osmolality and carbohydrate concentration [21].

Both of MRSA and *Pseudomonas aeruginosa* that have been reported as resistant pathogens for multiple antibiotics, were revealed stunning and surprising susceptibility for different types of honey. The results become more highlighted when are compared to that activity of tested antibiotic discs. This could be the best alternative drug to treat the multi resistant bacteria which has been presented by honey [23]. On the other hand, both MRSA and *Pseudomonas aeruginosa* showed resistance to the antibiotics which mean that there is no effectiveness from Amoxicillin & Ciprofloxacin (Tables 4 and 5). This generally in accordance with Hannan et al. Basson et al. [20,21,23]. Many researchers [12,13,22] have been reported that the inhibitory effect of honey could be due to osmotic effect, pH, hydrogen peroxide, etc. This inhibitory effect of honey might be decreased

with different types of honey depend on the condition of collection, time of maintenance, way that the honey was kept for long period, the effect of light & other conditions that might affect honey.

Regarding our results that show honey inhibitory activity against 6 types of bacteria especially those associated with multiple drug resistance, the assumption of topical application of honey as an antimicrobial agent for those patients suffer from surface infections such as ulcer, boils, injuries & surgical wounds.

CONCLUSION

Bacterial species that were collected from clinical specimens show obvious susceptibility against different local honey types that expressed clear inhibitory activity with different inhibition zone compared to that against Amoxicillin and Ciprofloxacin. *Pseudomonas spp*. and MRSA, the multi-drug resistant pathogens, exhibited responsiveness as the marked area of inhibitory honey activity appeared more than that with tested antibiotic discs. So, the strongly recommendation is that the medicinal use of honey as an antimicrobial agent should be highlighted and further in vitro and in vivo study of inhibitory activity of honey have to been conducted for determine Minimum Inhibitory Concentration (MIC).

CONFLICT OF INTEREST

None.

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