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In Vitro Antibacterial Activity of Some Natural and Trade Iraqi Honey against MRSA *Staphylococcus Heamolyticus* Isolated from Some Burned Patients in Misan City

Mohammed A. Abd Ali[1, *], Sailh M. Kidem[2], Ali A. Fadhil[3], Nusaybah KH.Saddam[3]

[1]College of Science, Misan University, Iraq
[2]College of Basic Education, Misan University, Iraq
[3]College of Science, Misan University, Iraq
*Corresponding author: waleed.abas22@yahoo.com

Abstract The study is aimed to evaluate the antibacterial activity of both natural Iraqi honeys (Sidr, Eucalyptus) and trade honey sample against MRSA *Staphylococcus heamolyticus* isolated from burned patients in alsader hospital-misan city then were analyzed between (October to December in 2015). The samples were taken in order to determine the bacterial profile and antibiotic susceptibility. The isolates of *Staphylococcus heamolyticus* were tested against 5 different antibiotics by a disk diffusion method 100% of the *Staphylococcus heamolyticus* isolates were resistant to the Optochin, Lincomycin, Ampicillin, Amphotercin, while the inhibitory effect of Amikacin antibiotic were (24mm) on MRSA *Staphylococcus heamolyticus*. Total antibacterial activity was evaluated by measuring the clear zone around the well. Honey samples were tested in different concentration (75%, 50 %, 25%, 10% and 100%). However, the results showed the absences of inhibitory zone to both natural and trade honeys type on MRSA *Staphylococcus heamolyticus* tested bacteria after dilution of(25% Sidr honey sample, 50% Eucalyptus honey sample, 50% Trade honey sample), while Sidr honey sample have more effective in 75%v/v concentration against MRSA *Staphylococcus heamolyticus* tested bacteria. The minimum inhibitory concentration (MIC) of the Sidr honey sample were also determined. Finally cytotoxicity evaluating toward human RBC, the results revealed the Iraqi honeys sample have not any cytotoxicity in all concentration. The good antimicrobial potency of sidr and Eucalyptus Iraqi honeys could potentially be used as therapeutic against MRSA *Staphylococcus heamolyticus* as an alternative to the costly antibiotics.

Keywords: *antibiotics, (MRSA) Staphylococcus heamolyticus, honey samples, (MIC) minimum inhibitory concentration, cytotoxicity*

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1. Introduction

Honey is an important and unique food product containing bioactive compounds derived from bees and plants [20]. Honey is produced from many sources and its antimicrobial activity. More recently, honey has been reported to have an inhibitory affect to around 60 species of gram – positives bacteria and gram – negatives bacteria [16]. Current evidence suggests that several factors may contribute to the antimicrobial properties of honey, the most important being osmolarity, acidity, the enzymatic generation of hydrogen peroxide and the presence of various non –peroxide compounds derived from the pollen or the nectar of flowers [24]. Honey possesses inherent antimicrobial properties due to high osmotic pressure with low water activity, in which the low water activity of honey is inhibitory to the growth of the majority of bacteria and many yeasts and moulds [17].The resistant strain, MRSA which is widespread, particularly in the hospital sitting. *S. aureus* (MRSA) generally remained an uncommon finding even in hospital sittings until the 1990s when there was an explosion in (MRSA) prevalence in hospitals of *S. aureus* (MRSA) [7].

The moist environment of chronic wounds is an ideal growth medium for bacteria [21], and infection is the prominent cause of delayed healing. This has become an increasing problem with the recent expansion of antibiotics – resistant bacteria [13]. Burns and chronic wound are particularly prone to infection with75% of dirty following burns involving infection [26]. One of the most emphasized subjects about pathogenesis of Staphylococci infections is the slime production characteristic [10]. Multi-resistant coagulase-negative Staphylococci (CoNS) may adhere to medical devices and surfaces through slime

which secretes out of the cell and has a mucopoly-saccaride stricture, and in this way, they may easily colonize and spread within hospital environment [27].

In a survey 51% *S. aureus* were found to be MRSA, as well as the bacteria cause food poisoning, toxic shock syndrome, scalded skin syndrome [15]. Antimicrobial resistance has become a global problem, The resistant strain MRSA which was first discovered in the UK in 1961.Now is widespread, particulary in hospital setting [9]. Burns are damage to the skin caused by a variety of non – mechanical sources including chemicals, electricity, heat, or nuclear radiation [22]. Burn wound infection is problematic because it delays healing. encourages scamming and may result in bacteremia, sepsis or multiple – organ dysfunction syndrome whereby organs from several systems are unable to maintain homeostasis on their own, requiring immediate medical attention [4].

The aim of this study is to evaluate the antimicrobial activity of some honey samples against MDR *Staphylococcus heamolyticus* compared with some antibiotics.

2. Material and Methods

The project was approved by the Department of biology in college of science /Misan university / Iraq.

2.1. Honey Samples

Thehoney sample used in this study was collected from Misan province / Iraq. It was collected in sterile container.

2.2. Isolation and Identification of *Staphylococcus Heamolyticus*

Staphylococcus heamolyticus were isolated from Burns patients. a sterile cotton swab was used. Samples were cultured onmanitol salt agar medium, incubated at 37°C overnight, the colony appeared then subcultured and purified. Identification of the isolates based on many characters such as colonial morphology, Gram stain, motility, oxidative, fermentation test, catalase citrate and oxidative tests, *Staphylococcus heamolyticus* isolates were stored in Nutrient broth [23].

2.3. Antibiotic Susceptibility Testing

Disc diffusion method test were done for all the isolated [8]. A suspension of each isolate was made at turbidity to 0.5 McFarland standard and then plated on to Muller – Hinton agar Plate. Antibiotic disc, was applied to each plate. The plates incubated at 37°C for 24h. After incubation the inhibition zone was measured, the results of all isolates compared with standard isolates of *Staphylococcus heamolyticus* were isolated from burned patients.

2.4. Antibiotic Susceptibility Assay

The well diffusion assay is suitable for aqueous honey samples because they are difficult to dry on paper discs [25]. A suspension of each isolate was made at turbidity equal to 0.5 McFarland standards and then plated on Muller- Hinton agar. Antibiotic disc was applied to each plate. The plate incubated at 37°C for 24 h. After incubation the inhibition zone was measured the results of all isolates compared with standard isolates of *Staphylococcus heamolyticus* were isolated from burned patients.

2.5. Preparation of Honey Samples

The honey sample was diluted by (1:1, 2:1, 4:1, 8:1, 16:1, 32:1) and non diluted honey (100%).

2.6. Determination of MIC by Agar Plate Dilution Method

According to the methods of [19], agar plate dilution test was used to determine the Minimum Inhibitory Concentration (MIC) of an antimicrobial agent.

Table 1. Antibiotics: fivetype of antibiotics used in this study

No	Antibiotic	Concentration	Company
1	Lincomycin	L10 (mcg)	Bioanalyse
2	Ampicillin	AM10 (mcg)	
3	Amikacin	AK30(mcg)	
4	Optochin	Op5(mcg)	
5	Amphotercin	AMC30(mcg)	

Table 2. showed Antibiotics sensitivity test of pathogenic *Staphylococcus heamolyticus* isolated from some patients in al sader hospital in misan city

No	Antibiotic	Concentration	Bacterial strain *Staphylococcus heamolyticus*
1	Lincomycin	L10 (mcg)	-
2	Ampicillin	AM10 (mcg)	-
3	Amikacin	AK30(mcg)	24mm
4	Optochin	Op5(mcg)	-
5	Amphotercin	AMC30(mcg)	-

Disk diameter (6mm).

Table 3. showed Antibacterial activity of three type of honey against MDR *Staphylococcus heamolyticus* compared with Optochin antibiotics

No	Honey type	Honey concentration	Inhibition zone (mm) *	Inhibition zone (mm) of Optochin antibiotics
1	Sidr Honey	100	18.5	ND
		75	20	
		50	14	
		25	6.5	
		10	6	
2	Eucalyptus Honey	100	12	ND
		75	7.5	
		50	6	
		25	6	
		10	6	
3	Trade Honey	100	7.5	ND
		75	10	
		50	6	
		25	6	
		10	6	

* mean of three value each number, ND=Not Done

Table 4. The mean of inhibition zone of Sidr honey sample against multi-drug isolated against *Staphylococcus heamolyticus*

Bacterial strain isolated	** The mean of inhibition zone of Sedar honey sample (mm)				
	100%⁄ml /ml	75 %⁄ml /ml	50%⁄ml /ml	25%⁄ml /ml	10%⁄ml /ml
Staphylococcus heamolyticus	18.5	20	14	6.5	6

*clinical strain ** mean of three value each number.

Table 5. The MIC of the of Sidr honey sample against multi-drug isolated against *Staphylococcus heamolyticus* isolated bacteriafrom clinical burn Patients

Sample	* Dilution of Sedar honey sample against multi-drug isolated against Staphylococcus haemolyticus						
	≥64	≥32	≥16	≥8	≥4	≥2	≥1
Staphylococcus heamolyticus	-	-	-	-	+	+	+

mean of three value each number.

Figure 1. Mueller Hinton agar media with antibiotic sensitive disc showing *Staphylococcus heamolyticus* resistant to 4 antibiotics

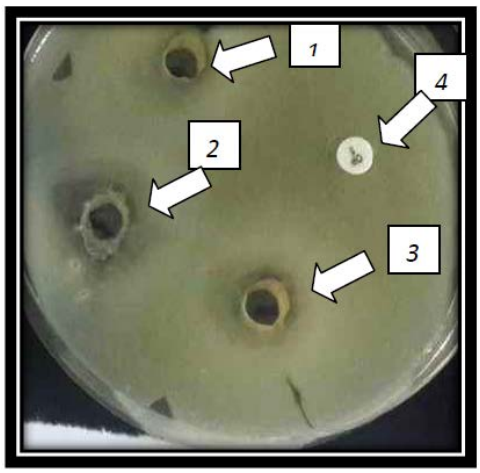

Figure 2. showing *Staphylococcus heamolyticus* resistant Optachin antibiotics (A) on blood agar media (B).on Mueller Hinton agar media with three type of honey [1 tride honey], [2 sedar honey], [3 Eucalyptus honey]

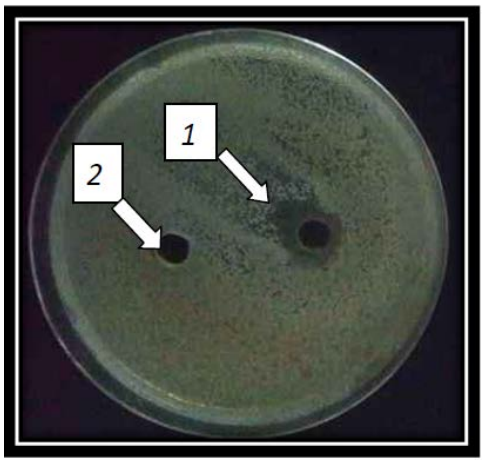

Figure 3. Mueller Hinton agar media with 100% V/ V sidr honey arrow1 and arrow 2 showen Distilled water

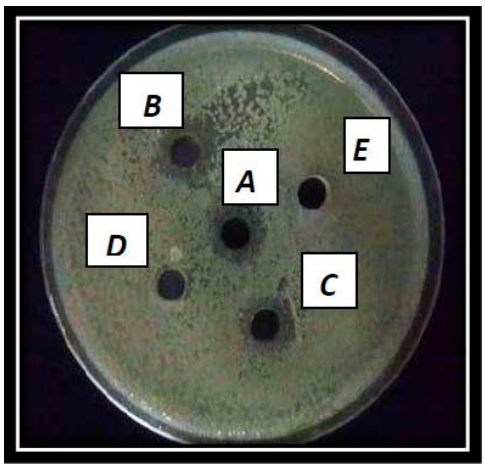

Figure 4. Mueller Hinton agar media with [100% V/ V sidr honey **A**] and [**B**75% V/ V sidr honey], [**C** and 50 % V/ V sidr honey], [**D** and 25% V/ V sidr honey], [**E** 10% V/ V sidr honey]

3. Results

Honey sample showed marked inhibition of growth on *Staphylococcus heamolyticus* isolated, the maximum inhibition zone was showed at concentration of 75% as 20 mm by Sidr honey sample, where minimum inhibition zone was showed at concentration of 25% as 6.5 mm.

[Table 2, Figure 1], showed high resistance to Lincomycin (100) and Optochin(100%), Ampicillin (100%), Amphotercin (100%). Multi-Drug resistance strain of *Staphylococcus heamolyticus* were recorded from clinical burn Patients. There was no resistance to Amikacin antibiotics.

All collected *Staphylococcus heamolyticus* isolated from clinical burn Patients were exposed to different

concentration of two honey Sample of natural sours and one trade sample. The results represented in [Table 3].

Honey sample showed marked inhibition of growth on *Staphylococcus heamolyticus* isolated, the maximum inhibition zonewas shown at concentration of 75% as 20 mm from sidr honey sample, which reduce to 14mm at 50% concentration [Table 3].

Showed that inhibition zone of *Staphylococcus heamolyticus* isolated bacteria increased as the concentration different concentration of two honey Sample and one trade sample, the susceptibility pattern to different concentration of two honey Sample isolated bacteria maximum inhibitory zone at 75 % v/v which was mean 20 mm but in low concentration which was mean 14mm against Multi – Drug isolated bacteria *Staphylococcus heamolyticus*.

In same time the results represented in [Table 3, Figure 2 A, B], showed the isolated bacteria *Staphylococcus heamolyticus* resistant Optachin antibiotics, showed three type of honey(arrow one trade honey inhibition zone), (arrow two sidr honey inhibition zone), (arrow three eucalyptus honey inhibition zone).

The results represented in [Table 3, Figure 3], showed arrow one distilled water and arrow two sider honey inhibition zone.

The results represented in [Table 5, Figure 4], showed the MIC of the sider honey recorded as (≥4V/V).

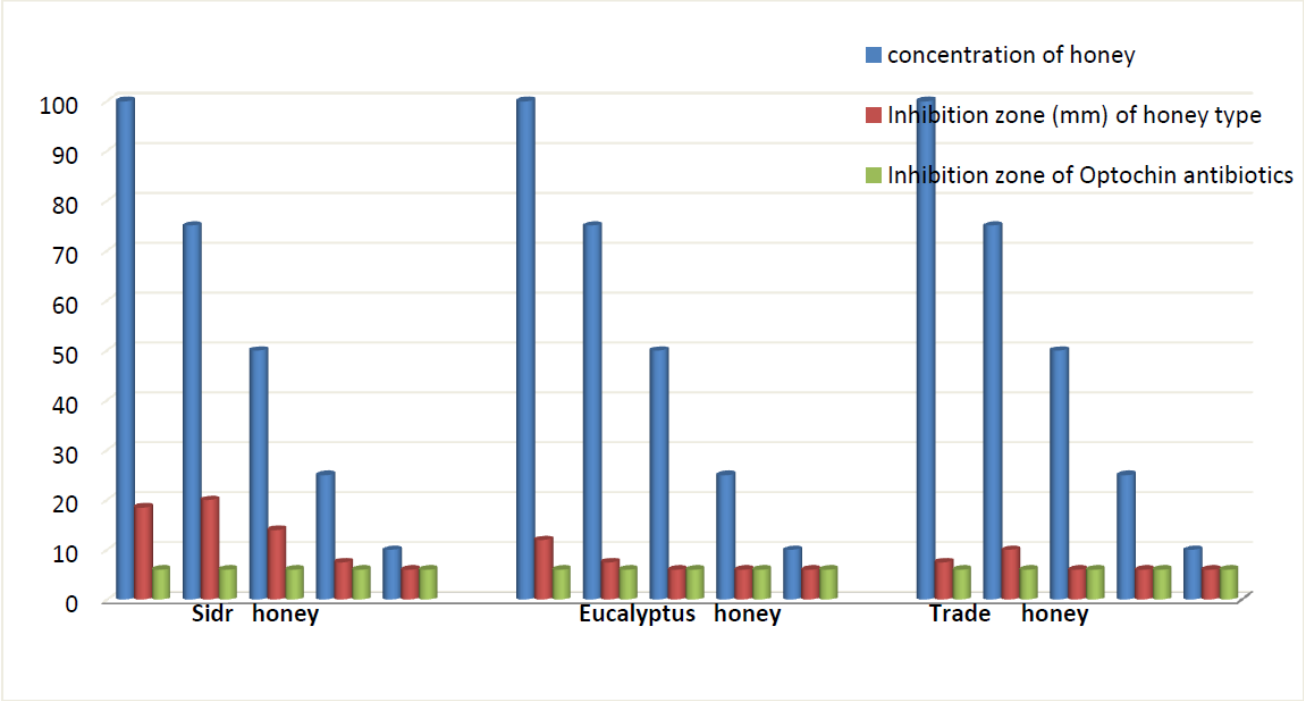

Figure 5. Antibacterial activity of three type of honey against MDR *Staphylococcus heamolyticus* compared with Optochin antibiotics

4. Discussion

Our study showed high prevalence of bacterial infection especially Coagulase-negative *Staphylococcus heamolyticus* are major cause of nosocomial and septicemia, especially for the patients who have immune deficiency and and malignancy, which can lead to morbidity and even mortality [14]. This study was focused on the susceptibility testing of clinically isolates belonging to Coagulase-negative *Staphylococcus heamolyticus* since these microorganisms are frequently isolated from burns patients in particular, as a cause of sepsis in patients. The relevant mechanism of penicillin resistace in *Staphylococcus heamolyticus* might be the patients with lowered affinity for beta – lactam antibiotics [5]. The results prevalence of *Staphylococcus heamolyticus* isolated and new resistant strains, the resistant was detected against Lincomycin (100%), Ampicillin (100%), Optochin (100%), Amphotercin(100%), Only Amikacin antibiotics showed Zone of inhibition as 24mm [Table 2, Figure 1], this results are agreement with [6].

There are four main mechanisms by which bacteria become resistant to antibiotics agents, Destruction and inactivation of the antibiotic by blockage of transport of the agent into the cell providing the cell with a replacement for the metabolic step inhibited by the drug, and protection of the target site by a bacterial protein therefore it is imperative to seek other sources of therapeutic agents [12]. which increased the inhibition zone against MDR *Staphylococcus heamolyticus* bacteria. Only Sidr honey sample of the three tested inhibited the growth of MDR *Staphylococcus heamolyticus* bacteria it is interesting that commercially available honey samples did not exhibit antibacterial properties. It is known that honey for sale (trade honey) can be heated from 45° to 80°C. Therefore, the loss of antibacterial activity in heat processed commercial (trade honey) sample could be accounted for denaturation of glucose oxidase [20]. The potency of sidr honey sample (75% concentration was found to be superior against *Staphylococcus heamolyticus* bacteria tested) these result is in agreement with [23].

Studies on the (MIC) of the honey samples on the test organism *Staphylococcus heamolyticus* bacteria showed that the low MIC were demonstrated (≥4 v/v) [Figure 4, Table 5], these result is in agreement with they showed

that honey have a greater MIC value were in range 5-20% (v⁄v), Our result were in agreement with [3], they found honey exhibited a fairly good antimicrobial activity against both Gram-positive and negative bacteria.

No inhibitory activity was detected when honeys were replaced by trade honey sample, suggesting that osmolaritydose not play an important role in growth inhibition. Thus, we can speculate that the observed activity is mainly due to the presence of antimicrobially active phytochemicls in the honey and the generation of hydrogen peroxide by the bee – derived enzyme glucose oxidase [2].

Finally a test was also carried out to examine the cytotoxicity assay by using [28], methods towards human red blood cells in which thethe deferent's honey sample, where found that they are not having any cytotoxicity an (1-500mg / ml). Results of this study suggest that the honey sample may be useful either alone or when combined with antimicrobial agents to treat (MDR *Staphylococcus heamolyticus* bacteria.

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