



Antibacterial Activity of Varying Honey Samples Against Some Bacteria in Libya

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(Received: 05 December 2025

Revised: 15 January 2026

Accepted: 10 February 2026)

KEYWORDS

Honey,
Staphylococcus aureus,
Klebsiella pneumoniae,
Antibiotics.

ABSTRACT:

The study aimed to evaluate the antibacterial efficacy of different honey samples against two types of pathogenic bacteria, where Four honey samples (Sidr honey and spring honey) were collected from two areas (Al-Marj and Al-Qubba). Honey samples showed an inhibitory effect at a 75% concentration against the various microorganisms studied, while a 25% concentration for both types of honey demonstrated weak activity against bacteria. In contrast, most of the tested antibiotics exhibited clear resistance to the two types of bacteria studied. While the antibiotic (CTR) proved ineffective against *K. pneumoniae*, *S. aureus* showed inconsistent resistance to both (CTR and Azm). This leads us to say that honey has a clear effect, making it a therapeutic alternative with nutritional and health value, and less costly for humans.

Introduction: The increasing resistance of bacteria to antibiotics has necessitated the search for new antimicrobial agents. The emergence of multidrug resistance is one of the most prominent current public health challenges, posing a threat to global health. (Abdelazeem Algammal *et al.* 2023). These resistant microorganisms can survive the spread of antibiotics, leading to ineffective treatment, which in turn leads to the spread of the disease. Antibiotics that were once useful against bacterial diseases are no longer effective in the current circumstances. (David, *et al.*, 2022). Additionally, the World Health Organization has described alternative medicines as an inexpensive means of achieving universal health coverage for populations around the world and has encouraged the use of complementary herbal remedies (Sacks, *et al.*, 1087). A variety of plants and their extracts have been used in treatments requiring antimicrobial activity, and one of the well-known natural antimicrobials described in ancient medicine was honey (Mandal & Manadal, 2011).

Objectives This study aims to compare the antibacterial efficacy of honey varieties from two regions in Libya against specific types of bacteria, as well as to balance it with antibiotics.

Methods: Honey samples (Sidr and spring) were collected from the Al-Qubba and Al-Marj regions of Libya. 25% and 75% concentrations of each variety were prepared. The study was conducted on two types of bacteria, Gram-positive and Gram-negative, which were cultured on nutrient agar using the concentration-saturated comb method. The cultures were incubated at 37°C for 18-24 hours, and the readings were recorded.

Results: Honey samples showed an inhibitory effect at a concentration of 75% against all studied microorganisms, while a 25% concentration of both honey types showed weak activity against bacteria. In contrast, the majority of tested antibiotics showed clear resistance to both types of bacteria studied. The antibiotic (CTR) was ineffective against *K. pneumoniae*, *S. aureus* showed inconsistent resistance to both (CTR and Azm).

Conclusions: This study showed that honey, in its various forms, exhibits high inhibitory activity against the tested bacteria, which may allow its use as an alternative to antibiotics, due to its content of polyphenols, 1, 2-dicarbonyl compounds, and hydrogen peroxide (Al-Masoudi, 2021). Antibiotics that were previously useful against bacterial diseases are no longer effective in the current situation (David *et al.*, 2022). The main source of this situation is the excessive consumption or misuse of pharmaceutical preparations with the aim of curing diseases faster rather than focusing on their effectiveness. Studies have also shown that the concentration of honey used, the nature of the isolated bacterial strain, the source of the honey, and the method of processing it are factors that affect its antibacterial activity (Kacaniova *et al.*, 2011).

1. Introduction

The increasing resistance of bacteria to antibiotics has necessitated the search for new antimicrobial agents. The

emergence of multidrug resistance is one of the most prominent current public health challenges, posing a threat to global health. (Abdelazeem Algammal *et al.* 2023). According to the World Health Organization, the



effectiveness of antibiotics is declining due to resistance in microorganisms, especially to synthetic antibiotics. These resistant organisms can survive in the face of widespread antibiotic resistance, resulting in ineffective treatment, which in turn leads to disease spread. Antibiotics that were once effective against bacterial diseases are now largely useless (David, *et al.*, 2022). The leading source this scenario involves the overuse or misuse of pharmaceutical drugs to Treat diseases more quickly rather than focusing on their effectiveness. Furthermore, spontaneous evolution, bacterial mutations, and the transfer of resistance genes via horizontal gene transfer are also key elements in antimicrobial resistance. (Dadgostar, 2019). In addition, the World Health Organization described alternative therapies as an inexpensive way to achieve the goal of universal health coverage for the world's population and encouraged the use of alternative herbal medicines. (Sacks, *et al.*, 1087).

A variety of plants and their extracts have been used for treatments requiring antimicrobial efficacy, and one of the well-known natural antibiotics prescribed in ancient medicine is honey. (Mandal & Manadal, 2011). Though honey is widely used in traditional medicine, its use in modern medicine is limited (Greenwood, 1993). A natural agent that has been used for long periods for therapeutic purposes is honey, due to its phytochemical properties (Zammit Young & Blundell, 2023), antibacterial properties (Mandal & Mandal, 2011), antioxidant properties (Ahmed *et al.* 2018), and anti-inflammatory properties (Silva *et al.* 2021). Moreover, honey is used to treat various inflammations and is considered effective as a dressing for wounds, including surgical wounds, burns, and skin ulcers. This is mainly because it accelerates the growth of new tissue, promotes wound healing, and relieves pain (Lusby *et al.*, 2002).

2. Methods

Sample Collection: Four honey samples (Sidr honey and spring honey) were selected from two regions (Al-Marj and Al-Qubba): Al-Qubba is distinguished by the symbol (Q), and Al-Marj by the symbol (M).

Tested Bacterial Samples: Two types of bacteria were used in this study: Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Klebsiella pneumoniae*). The organisms examined were

obtained from (Source: Microbiology Laboratory, Omar Al-Mukhtar University, Al-Bayda).

Antibiotic Discs: Ciprofloxacin (CIP10mcg), Ceftriaxone (CTR 10mcg) and Azithromycin (AZM 15mcg).

Preparation of Honey Solutions: In this study, different concentrations (25% and 75%) of each honey sample were carefully prepared. 0.75 ml of honey was diluted in 0.25 ml of sterile water to make a 75% honey solution (v/v). Similarly, to prepare a 25% concentration, 0.25 ml of honey was diluted in 0.75 ml of sterile water sequentially, respectively, (Mama *et al.*, 2019). In addition to testing for some antibiotics.

Testing the Inhibitory Activity of Honey: The sensitivity method (disc diffusion method) was used. (Filtered paper discs) were obtained and saturated with varying concentrations of honey. The tested bacteria were spread onto nutrient agar plates using a sterile swab, and the plates were left at room temperature for inoculation. Discs immersed in honey dilutions were then placed on top, along with antibiotic discs. The plates were incubated at 37°C for 18–24 hours in the Botany Department Laboratory, University of Derna, Al-Qubba.

3. Results and Discussion

Based on the results shown in the tables (1, 2, 3 &4), it was experiential that all tested honey concentrations had varying degrees of inhibition on bacterial growth. A 25% concentration of both types of honey showed weak activity against bacteria compared to a 75% concentration. In contrast, the majority of the tested antibiotics showed clear resistance to both types of bacteria examined. While the antibiotic CTR was ineffective against *Klebsiella pneumoniae*, *Staphylococcus aureus* showed varying resistance to both CTR and Azm, as detailed in the tables. These results are consistent with the study by (Abdel-Aal *et al.*) A 25% concentration of both types of honey showed weak activity against bacteria compared to a 75% concentration. In contrast, the majority of the tested antibiotics showed clear resistance to both types of bacteria examined. While the antibiotic CTR was ineffective against *Klebsiella pneumoniae*, *Staphylococcus aureus* showed varying resistance to both CTR and Azm, as detailed in the tables. These results are consistent with the study by Abdel-Aal *et al.*,



(2007), which indicated that honey had a more pronounced inhibitory effect (85.7%) on Gram-negative bacteria (*Pseudomonas aeruginosa*, *Enterobacter spp.*, *Klebsiella*). A 100% inhibition was observed in the case of MRSA methicillin-resistant (*Staphylococcus aureus*) compared to antibiotic use alone.

Table 1. The inhibitory effect of two types of honey (Sidr and Spring) on *Staphylococcus aureus* bacteria in the Al-Qubba area is shown, in conjunction with tested antibiotics.

Concentrate + Type of honey	<i>S. aureus</i>	Antibiotic		
		CIP	CTR	AZM
QR (25%)	-	+	+	+
QR (75%)	+	+	-	-
QS (25%)	+	+	+	+
QS (75%)	+	+	+	+

Table 2. The inhibitory effect of two types of honey (Sidr and Spring) on *Staphylococcus aureus* bacteria in the Marj area is demonstrated in conjunction with tested antibiotics.

Concentrate + Type of honey	<i>S. aureus</i>	Antibiotic		
		CIP	CTR	AZM
MR (25%)	-	+	+	+
MR (75%)	+	+	+	+
MS (25%)	+	+	+	+
MS (75%)	+	+	+	+

Table 3. The inhibitory effect of two types of honey (Sidr and Spring) on bacteria *Klebsiella pneumoniae* in the Al-Qubba area is demonstrated. In conjunction with tested antibiotics.

Concentrate + Type of honey	<i>K.pneu moniae</i>	Antibiotic		
		CIP	CTR	AZM
QR (25%)	+	+	-	+
QR (75%)	+	+	-	+
QS (25%)	+	+	-	+
QS (75%)	+	+	-	+

Table 4. The inhibitory effect of two types of honey (Sidr and Spring) on bacteria *Klebsiella pneumoniae* in the Al-Marj area is demonstrated. In conjunction with tested antibiotics

Concentrate + Type of honey	<i>K. pneumoniae</i>	Antibiotic		
		CIP	CTR	AZM
MR (25%)	-	+	-	+
MR (75%)	+	+	-	+
MS (25%)	+	+	-	+
MS (75%)	+	+	-	+

(+): Resistance or Inhibition, (-): Non-resistance or Noninhibition, (QR): Spring honey al-Qubba, (SQ): Sidr honey al-Qubba, (MR): Spring honey al-Marj, (SM): Sidr honey al-Marj, (CIP): Ciprofloxacin, (CTR): Ceftriaxone, (AZM): Azithromycin.

The inhibitory activity of honey is attributed to several factors such as osmotic pressure, low acidity, high sugar content, and hydrogen peroxide formation (Weston, 2000), the presence of phenolic acids (Alvarez, *et al.*, 2010), lysozymes (Israili, 2013), flavonoids (Kwakman & Zaat, 2011), and more recently, the presence of methylglyoxal ide defensin-1 (bee antibacterial peptide) as an important antibacterial compound in honey (Israili, 2013). Value variation in composition also emerges in honeys derived from the same plant source, and this is a result of seasonal climate changes or from a different geographical origin (Lugomer *et al.*, 2017). Furthermore, there are significant variations in the antimicrobial efficacy of certain types of honey, and it has been shown that honey of the same type but from a different geographical origin has exhibited significant differences in antibacterial activity (kalaba, *et al.*, 2020). While Allen *et al.*, (1991) It has been shown that there are many varieties of honey with and without antibacterial properties, and it has been assumed that the type of flower that forms the source of the nectar determines the nature of the antibacterial activity of the honey. Studies have also shown that the concentration of honey used, the nature of the bacterial strain, the source of the honey, and the method of processing it are factors that affect its antibacterial activity (Kacaniova *et al.*, 2011).



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