# *In Vitro* Antibacterial Activity of Honey against Clinical Isolates Associated With Urinary Tract Infections

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## ABSTRACT

Honey has been used as a traditional remedy for microbial infections since ancient times. With increasing concerns about antibiotic resistance, there is growing interest in evaluating natural products like honey for their antimicrobial properties. This study investigates the antibacterial activity of honey against *Staphylococcus aureus* and Escherichia coli, two common pathogens responsible for urinary tract infections (UTIs). Honey samples were obtained from local commercial producers in Bauchi State, Nigeria. The samples were diluted to concentrations of 25%, 50%, 75%, and 100% (v/v). Clinical isolates of *S. aureus* and *E. coli* were obtained from UTI patients and confirmed using standard microbiological techniques. Antibacterial activity was assessed using the agar well diffusion method. Mueller-Hinton agar plates were inoculated with standardized bacterial suspensions, and wells were filled with different honey concentrations. Gentamicin (10µg) and Ofloxacin (5µg) were used as control antibiotics. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured. The undiluted honey (100% concentration) exhibited the highest antibacterial activity against all tested bacteria. The inhibition zones ranged from 16–24 mm for *S. aureus* and 19–22 mm for *E. coli* across different honey concentrations. The antibacterial activity of honey was superior to Gentamicin but comparable to ofloxacin. Statistical analysis showed no significant difference ($P>0.05$) between the mean inhibition zones at different honey concentrations. The tested honey demonstrated antibacterial activity against S. aureus and E. coli isolates from UTIs. These findings suggest that honey could serve as an alternative treatment for bacterial infections, particularly in the face of rising antibiotic resistance.

## INTRODUCTION

Honey, a natural sweet substance produced by bees, has been utilized for centuries due to its numerous medicinal properties. It is formed when bees collect nectar and other plant deposits, enzymatically modify them, and store the final product in honeycombs [1]. Honey is rich in enzymatic and non-enzymatic antioxidants, including catalase, ascorbic acid, flavonoids, and alkaloids [2]. Historically, Egyptians and Greeks used honey for wound care due to its high osmolarity, which inhibits bacterial growth and promotes healing [3]. Honey contains various compounds, including sugars, proteins, amino acids, enzymes, organic acids, vitamins, minerals, phenolic compounds, and volatile substances. It remains stable even when exposed to heat or stored for extended periods [3]. Historical evidence suggests that honey was used for wound treatment as early as 4,500 years ago, as documented in ancient inscriptions [4].

The use of honey as a traditional remedy for microbial infections dates back to ancient civilizations, with numerous studies validating its antimicrobial potential. Manuka honey, in particular, has been widely researched and found to be effective against a range of pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) [5], [6]. Recent research in Pakistan has presented honey as a supplement for antibacterial resistance [7]. However, the antibacterial activity of honey varies depending on its botanical origin and the presence of bioactive compounds [8]. With the increasing recognition of honey's potential as a topical agent in wound care, it is imperative to assess different honey types for their therapeutic efficacy [9]. A recent study in

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Europe identified *Staphylococcus aureus* and *Escherichia coli* as common and diverse pathogens in clinical settings, often linked to healthcare-associated infections. *S. aureus*, a Gram-positive coccus, is known for its ability to cause a wide range of infections, from minor skin issues to life-threatening diseases, with strains like methicillin-resistant *Staphylococcus aureus* (MRSA) posing significant treatment challenges [10]. On the other hand, *E. coli*, a Gram-negative bacillus, is typically harmless in the intestines but, in certain strains, can lead to serious infections such as urinary tract infections and sepsis [11].

Given the global rise in antibiotic resistance, the search for effective alternatives is urgent. Unlike antibiotics, honey has not been associated with microbial resistance, making it a promising option for antimicrobial therapy [12]. The challenge of bacterial infections and antimicrobial resistance remains a global health concern. Chemotherapy and antibiotic treatments have been the primary approaches for managing bacterial infections, but their efficacy has been undermined by the rapid emergence of antibiotic-resistant strains. The overuse of antibiotics has led to widespread resistance, necessitating the search for alternative antimicrobial agents [13].

However, current evidence on honey's antimicrobial efficacy must address scientific concerns regarding its effectiveness in combating antimicrobial resistance. Many studies have involved small sample sizes or have been conducted as last-resort treatments when standard therapies fail [14], [15]. This study aims to assess the antibacterial activity of honey against selected clinical isolates. Specifically, it seeks to analyze and identify the bacterial isolates and determine the optimal dilution levels at which honey maintains its antimicrobial properties. The findings from this research could provide a scientific basis for incorporating honey as a viable alternative to conventional antibiotics in managing bacterial infections.

## MATERIALS AND METHODS

### Source of Honey

The honey used in this study was sourced from 3 different source but the locally extracted honey from commercial producers in Bauchi State, Nigeria was selected purposively after passing the honey potency test. The honey was unadulterated, containing no diluents or additives, and had not been subjected to any heating process. The honey was characterized by its high viscosity, moderately thick consistency, mild scent, and light coloration. (ref?)

### Source of Isolates

The bacterial isolates utilized in this study were obtained from the Medical Laboratory Department of the Federal Medical Center (FMC), Azare, Bauchi State. The confirmed stock cultures of *Staphylococcus aureus* and *Escherichia coli*, both isolated from urinary tract infections (UTIs), were used. Confirmatory tests, including Gram staining for differentiation of Gram-positive and Gram-negative bacteria, as well as biochemical tests such as Indole, Catalase, and Coagulase, were performed at the Microbiology Laboratory of Bauchi State University Gadau (BASUG)? Now SAZU?

### Processing of Honey Samples

The honey samples were aseptically filtered using a sterile mesh to remove any debris. The filtered honey was referred to as "neat." The neat honey was then diluted with sterile distilled water to prepare concentrations of 25%, 50%, 75%, and 100% (v/v). The preparation of each concentration was as follows:

25% (2.5 mL honey + 7.5 mL distilled water)

50% (5 mL honey + 5 mL distilled water)

75% (7.5 mL honey + 2.5 mL distilled water)

100% (neat honey without dilution).

These preparations followed the method described by El jack *et al.* (2014).

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**Preparation of 0.5 McFarland Standards and Bacterial Standardization**

A 1% (v/v) solution of sulfuric acid was prepared by adding 1 mL of concentrated sulfuric acid to 99 mL of distilled water and mixed thoroughly. A 1% (w/v) solution of barium chloride ($BaCl_2 \cdot 2H_2O$) was prepared by dissolving 0.5 g of dehydrate barium chloride in 50 mL of distilled water. To prepare the McFarland standard, 0.6 mL of the barium chloride solution was added to 99.4 mL of the sulfuric acid solution and mixed. A small volume of this turbid solution was transferred into a capped tube, as described by Cheesbrough (2000).

**Antibacterial Sensitivity Testing**

The McFarland No. 5 standard was prepared as described by Cheesbrough (2000). Clinical isolates of Staphylococcus aureus and Escherichia coli were inoculated and spread onto nutrient agar plates and incubated for 18-24 hours. Two to three colonies of each isolate were picked using a sterile wire loop, transferred to sterile saline solution, and the turbidity was adjusted to the 0.5 McFarland standard (equivalent to $1.5 \times 10^8$ CFU/mL). Each nutrient agar plate was evenly inoculated with a sterile swab dipped into the bacterial suspension. Wells of equal distance (6 mm in diameter) were bored into the agar medium using a sterile cork borer. Fifty microliters (50 µL) of honey samples, at concentrations of 25%, 50%, 75%, and 100%, were introduced into the wells. A positive control well was filled with sterile distilled water. The plates were incubated at 37°C for 18-24 hours, and the inhibition zones, represented by clear areas around the wells, were measured in millimeters (mm) using a meter rule. All experiments were conducted in triplicate, and the average results were recorded.

**Antibiotic Susceptibility Testing**

The bacterial isolates were also tested for their susceptibility to common commercial antibiotics (Gentamicin and Ofloxacin) using the same method employed for testing the antibacterial activity of honey. The sensitivity of the isolates was determined by observing zones of inhibition around antibiotic discs.

**Statistical Analysis**

The antibacterial effects of honey, expressed as mean inhibition zones ± standard deviation (SD), were compared using descriptive statistics. Data analysis was performed using Microsoft Office Excel 2003. The means and standard deviations of the triplicate analyses were calculated, and the differences between means were assessed using analysis of variance (ANOVA). A post hoc test was conducted when the F-test revealed significant differences, with significance defined as a p-value of $< 0.05$.

**Ethical Clearance:** Ethical approval for this study was granted by the ministry of health Institutional Review Board (Reference No. MOH/ADH/ADM621/V.I/431) on September 24, 2023.

## RESULTS

This section presents the results of the antibacterial activity of honey (Apis mellifera) against clinical isolates of Staphylococcus aureus and Escherichia coli. The honey was tested at four different concentrations (25%, 50%, 75%, and 100%) and compared with two commercially available antibiotics, Gentamycin (10 µg) and Ofloxacin (5 µg), to evaluate its efficacy. The results from the tables provide insights into the inhibitory effect of honey at varying concentrations compared with the standard antibiotics.

**Table 4.1: Zones of Inhibition (mm) of Honey (Apis mellifera) on Clinical Isolates at Different Concentrations**

Table 4.1 presents the antibacterial activity of honey (Apis mellifera) at different concentrations against Staphylococcus aureus and Escherichia coli. The zones of inhibition were measured in millimeters (mm) for each concentration (25%, 50%, 75%, and 100%) and replicated three times. The data show that honey exhibited inhibitory effects on both bacterial strains, with varying degrees of effectiveness depending on the concentration.

For Staphylococcus aureus, the highest inhibition was observed at the 25% concentration, with a mean inhibition zone of 24 mm. However, as the concentration increased, the inhibitory effect decreased, with the mean zone

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measuring 16 mm at 100% concentration. For Escherichia coli, the inhibition zones varied more consistently across concentrations, with the mean inhibition zone ranging from 19 mm at 25% to 22 mm at 100%. This suggests that honey was more consistently effective against *Escherichia coli* than *Staphylococcus aureus* at higher concentrations.

Table 4.2: Antibiogram of Commonly Used Commercial Antibiotics Against the Selected Clinical Isolate as Control

| Test Bacteria | No. of Replicates (R) | Concentration 25% | Concentration 50% | Concentration 75% | Concentration 100% |
|---|---|---|---|---|---|
| *Staphylococcus aureus* | R1 | 21 | 20 | 28 | 17 |
| *Staphylococcus aureus* | R2 | 24 | 19 | 11 | 15 |
| *Staphylococcus aureus* | R3 | 27 | 15 | 12 | 16 |
| Mean | | 24 | 18 | 17 | 16 |
| *Escherichia coli* | R1 | 10 | 24 | 22 | 21 |
| *Escherichia coli* | R2 | 25 | 20 | 20 | 25 |
| *Escherichia coli* | R3 | 23 | 19 | 15 | 20 |
| Mean | | 19 | 21 | 19 | 22 |

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Table 4.2 presents the comparison of the inhibition zones of commonly used commercial antibiotics, Gentamycin (10 µg) and Ofloxacin (5 µg), against Staphylococcus aureus and Escherichia coli. The results show that both antibiotics exhibited stronger antibacterial activity compared to honey. Ofloxacin had the widest inhibition zone for both bacterial strains, with inhibition zones of 22 mm for Staphylococcus aureus and 21 mm for Escherichia coli Gentamycin also displayed some inhibitory effect but was less potent, with inhibition zones of 16 mm for Staphylococcus aureus and 15 mm for Escherichia coli. This suggests that while honey possesses some antibacterial properties, its activity is less potent than that of the commercial antibiotics tested.

Table 4.3: The Mean ± Standard Error of Honey at Different Concentrations Compared with Antibiotics as Control

| **Test Bacteria** | **Antibiotics (µg)** | **Minimum Zone of Inhibition (mm)** | **Total Antibiotics** |
|---|---|---|---|
| Staphylococcus aureus | Gen 10µg | 16 | |
| Staphylococcus aureus | Ofl 5µg | 22 | |
| Escherichia coli | Gen 10µg | 15 | |
| Escherichia coli | Ofl 5µg | 21 | |
| | | | 18 |

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Table 4.3 provides a summary of the mean inhibition zones of honey at various concentrations, along with the standard error, compared to the control antibiotics (Gentamycin and Ofloxacin). The results show the variation in honey's effectiveness against Staphylococcus aureus and Escherichia coli at different concentrations.

For Staphylococcus aureus, honey at 25% concentration had the highest mean inhibition zone of 24 mm (±1.73). However, the inhibitory effect decreased as the concentration increased, with the zone measuring only 16 mm (±0.50) at 100%. For Escherichia coli, honey showed more consistent inhibition across concentrations, with a slight increase in inhibition zone at 100% (22 mm ±1.53). The standard antibiotics, Gentamycin (10 µg) and Ofloxacin (5 µg), produced inhibition zones of 16 mm and 22 mm, respectively

**Analysis of variance (ANOVA) single factor for zone of inhibition of selected clinical organisms.**

| Clinical Isolates | Honey 25% | Honey 50% | Honey 75% | Honey 100% | GEN10µg | OFL5µg |
|---|---|---|---|---|---|---|
| *S.aureus* | 24 ± 1.73 | 18 ± 1.52 | 11 ± 0.50 | 16 ± 0.50 | 16 | 22 |
| *E. coli* | 19 ± 4.71 | 21 ±1.52 | 22 ± 1.50 | 22 ± 1.53 | 15 | 21 |

The analysis of variance (ANOVA) single-factor test was conducted to evaluate the differences in the mean zones of inhibition (measured in mm) of Apis mellifera honey on selected clinical isolates at varying concentrations. The dataset consisted of eight groups, each with three replicates, and their respective sum, average, and variance values were calculated.

The ANOVA results indicated that the between-group sum of squares (SS) was 163.17, with a mean square (MS) of 23.31. The within-group sum of squares was 390.67, resulting in an MS of 24.42. The computed F-value (0.95) was found to be lower than the critical F-value (2.66) at a 5% significance level. The corresponding p-value (0.494) exceeded the threshold of 0.05, indicating that there was no statistically significant difference in the mean zones of inhibition across the different concentrations of honey.

| Groups | Count | Sum | Average | Variance |
|---|---|---|---|---|
| Column 1 | 3 | 72 | 24 | 9 |
| Column 2 | 3 | 54 | 18 | 7 |
| Column 3 | 3 | 51 | 17 | 91 |
| Column 4 | 3 | 48 | 16 | 1 |
| Column 5 | 3 | 58 | 19.33333 | 66.33333 |
| Column 6 | 3 | 63 | 21 | 7 |
| Column 7 | 3 | 66 | 22 | 7 |
| Column 8 | 3 | 66 | 22 | 7 |

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| Source of Variation | SS | Df | MS | F | P-value | F crit |
| Between Groups | 163.1667 | 7 | 23.30952 | 0.954656 | 0.49437 | 2.657197 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Within Groups | 390.6667 | 16 | 24.41667 | | | |
| Total | 553.8333 | 23 | | | | |

## DISCUSSION

This study investigates the in vitro antibacterial activity of honey against clinical isolates associated with urinary tract infections. The results demonstrated that honey has the potential to inhibit the growth of both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Our findings are consistent with a study in Europe, which reported that honey inhibited the growth of *S. aureus*, *E. coli*, and *Pseudomonas* sp. [16]. A study in Northern Saudi Arabia showed that the efficacy of honey depends on its source and type [17]. Moreover, a study in Asia also found that honey exhibited substantial antimicrobial activity against both Gram-negative and Gram-positive bacteria, with significant activity observed against *Pseudomonas aeruginosa* and *S. aureus* [12], [18].

The results of this study also show antimicrobial activity against *S. aureus*, with honey being effective across varying concentrations. This study result is consistent with a study at the University of Waikato, New Zealand, which identified *S. aureus* as one of the most susceptible bacterial species to the antibacterial effects of honey [19]. The observed antibacterial activity can be attributed to several factors, including the osmotic effect, low pH, and the presence of hydrogen peroxide in honey, which acts as an inhibitory factor [20].

In this study the antimicrobial potency of honey was particularly evident against *Pseudomonas* species, a finding that aligns with research in India, which observed significant antimicrobial activity of honey against *P. aeruginosa* and *E. coli*. Table 4.3 shows the percentage sensitivity of bacterial strains, with *P. aeruginosa* demonstrating the highest sensitivity at 100%, followed by *E. coli* at 96.4%. These results further support the notion that honey is an effective agent against certain resistant bacterial strains, particularly *Pseudomonas* [21].

Interestingly, the activity of Gentamicin was found to be less potent than undiluted honey or any of its aqueous dilutions. This finding is consistent with studies in Saudi Arabia and Greece, which demonstrated that honey had a stronger inhibitory effect on Gram-negative bacteria, including *P. aeruginosa* and *Enterobacter* spp. Studies have observed that Gram-negative bacteria are generally more sensitive to the antimicrobial actions of honey than Gram-positive bacteria [22], [23].

The antimicrobial effect of honey has also been shown to be particularly effective against *Pseudomonas* and *Acinetobacter* species, which are resistant to antibiotics such as Gentamicin, Ceftriaxone, Amikacin, and Tobramycin [24]. Similarly, a study in Egypt demonstrated that strains of *P. aeruginosa* resistant to higher antibiotics were still sensitive to the antibacterial effects of honey [25].

Previous studies have attributed the antimicrobial effects of honey against Gram-negative bacteria to various factors, including the high content of tetracycline derivatives, hydrogen peroxide, powerful antioxidants, a naturally low pH, phenolic acids, lysozyme, and flavonoids. These factors collectively contribute to the antibacterial potency of honey [20], [25].

The Minimum Inhibitory Concentration (MIC) of honey was found to be less than 10% against *P. aeruginosa*, which aligns with a study in the United Kingdom on the MIC of Manuka honey, which had an MIC of less than 10% against 17 strains of *P. aeruginosa*. Honeys with an MIC of 10-20% are expected to be effective in preventing bacterial growth, with *P. aeruginosa* being particularly susceptible, followed by *E. coli* and *S. aureus* [26].

**Limitation**: This study was limited by the use of only two bacterial species, *Staphylococcus aureus* and *Escherichia coli*, and a single honey sample, limiting generalizability. Additionally, the study was conducted in vitro, and the potential in vivo effects of honey were not explored. Variations in honey composition due to floral source or processing methods were not considered. The study also did not investigate long-term effects, bacterial resistance, or the exact mechanisms by which honey exerts its antimicrobial activity. Lastly, honey's storage conditions were not assessed.

## CONCLUSION

In conclusion, this study demonstrates that honey possesses significant antibacterial activity against both Gram-positive and Gram-negative bacteria, including S. aureus and E. coli. The antibacterial potency of honey varies with concentration, with the highest activity observed at 100% concentration. The study highlights the potential of honey as a natural alternative to antibiotics, particularly for treating infections caused by resistant strains. However, the variability in honey's antibacterial activity due to factors such as the honey's source and composition emphasizes the need for caution in predicting its efficacy.

Based on these findings, honey can be considered a promising adjunct for treating urinary tract infections and other bacterial infections, but further studies are required to fully explore its therapeutic potential.

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