# Comparative Evaluation of the Antibacterial Efficacy of Bee Honey and Commercial Antibiotics Against Clinically Relevant Bacterial Pathogens

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**ABSTRACT:**

This study evaluated the antibacterial activity of bee honey against five common bacterial pathogens associated with human infections: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. Different concentrations of bee honey were tested for their antimicrobial efficacy, and varying zones of inhibition were observed against the selected bacterial pathogens. Among the concentrations examined, 20% bee honey exhibited the highest antibacterial activity, demonstrating significant inhibitory effects against the tested microorganisms.

The antimicrobial properties of bee honey can be attributed to the presence of various bioactive secondary metabolites. Previous studies, including those reported by( Banerjee *et al*.,2022 )have demonstrated that honey contains phytochemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, and steroids. These secondary metabolites play a crucial role in the natural defense mechanisms of honey against microorganisms and other biological threats. Their antimicrobial effects are exerted through multiple mechanisms, including disruption of microbial cell structures, inhibition of essential metabolic processes, and interference with microbial growth and proliferation. The findings of this study support the potential use of bee honey as a natural antibacterial agent against common bacterial pathogens.

**INTRODUCTION**:

The rapid emergence of antimicrobial resistance (AMR) has become a significant global health concern, compromising the effectiveness of existing antibiotics and necessitating the development of alternative antimicrobial agents **(**Banerjee *et al.*, 2022). Concerns over the toxicity, adverse effects, and environmental impact of synthetic drugs have further stimulated interest in natural products, which are rich sources of bioactive compounds such as alkaloids, flavonoids, glycosides, steroids, and saponins with well-documented medicinal and pharmacological properties (Cushnie TPT, Lamb AJ *et al.*, 2005)

The increasing prevalence of multidrug-resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, has intensified the search for novel antimicrobial agents. Natural products have long been recognized for their broad-spectrum antimicrobial activity, lower toxicity, and diverse mechanisms of action, making them promising candidates for the development of new therapeutic agents .

Honey has attracted considerable attention as a natural antimicrobial agent because of its broad-spectrum antibacterial activity. Its antimicrobial effects are attributed to multiple factors, including high osmolarity,

acidic pH, hydrogen peroxide production, methylglyoxal, phenolic compounds, and antimicrobial peptides such as bee defensin-1, which collectively inhibit the growth of a wide range of microorganisms, including antibiotic-resistant bacteria. Given the increasing challenge of antibiotic resistance, honey represents a promising alternative therapeutic agent, warranting further investigation to elucidate its bioactive components, mechanisms of action, and potential clinical application **(**Kwakman PHS, Zaat SAJ *et al.,2005).*

## METHODOLOGY:

### Study Design and Setting

A laboratory-based experimental study was conducted in the Department of Microbiology, MMM College of Health Sciences, Mogappair East, Chennai, Tamil Nadu, India, over a period of three months from January to March 2020. The study aimed to evaluate and compare the antibacterial activity of bee honey against selected clinically important bacterial pathogens.

### Collection and Storage of Bee Honey

Fresh, unprocessed bee honey was obtained from a certified local honey supplier in Thiruvallur, Tamilnadu, India. The honey samples were collected in sterile, airtight glass containers and stored at room temperature, protected from direct sunlight, until further analysis.

Five clinically relevant bacterial pathogens commonly implicated in human infections were included in the study: *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Proteus mirabilis.* Stock cultures were procured from a tertiary care hospital microbiology laboratory and were authenticated using standard microbiological procedures, including colony morphology, Gram staining, and biochemical characterization.

The bacterial isolates were initially cultured in Nutrient Broth and incubated at 37°C for 24 hr. Subsequently, the cultures were subcultured onto Nutrient Agar plates and maintained at 4°C until use. Fresh subcultures were prepared periodically to ensure viability and preserve the phenotypic characteristics of the organisms .

### Preparation of Honey Dilutions

Honey solutions of three different concentrations (5%, 10%, and 20% v/v) were prepared using sterile distilled water. The required volume of honey was aseptically diluted with distilled water to obtain a final volume of 100 ml. All dilutions were freshly prepared prior to antimicrobial testing.

### Culture Media

The microbiological media used in this study included Nutrient Broth (NB), Nutrient Agar (NA), and Mueller–Hinton Agar (MHA) for the cultivation and antimicrobial susceptibility testing of bacterial isolates. Selective and differential media were employed for the confirmation of the test organisms, with Mannitol Salt Agar (MSA) used for *Staphylococcus aureus,* Sheep Blood Agar for *Streptococcus pyogenes,* MacConkey Agar for *Klebsiella pneumoniae,* CLED Agar for *Pseudomonas aeruginosa,* and Brain Heart Infusion Agar for *Proteus mirabilis*. All culture media were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C under 15 psi pressure for 15 minutes before use.

### Maintenance and Preservation of Bacterial Cultures

The bacterial isolates were maintained on Nutrient Agar slants and stored at 4°C throughout the study period. Routine subculturing was performed at regular intervals to maintain culture viability and purity.

**Identification of Bacterial Isolates**

The bacterial isolates were identified and confirmed using conventional microbiological methods, including microscopic examination, cultural characteristics, and biochemical tests.

**Gram Staining**

Gram staining was performed to determine the Gram reaction and cellular morphology of the bacterial isolates. Smears were prepared from pure cultures, heat-fixed, stained with crystal violet, treated with Gram's iodine, decolorized with ethanol, counterstained with safranin, and examined microscopically under oil immersion.

**Biochemical Characterization**

The bacterial isolates were characterized through standard biochemical assays, including the catalase, indole, methyl red (MR), Voges–Proskauer (VP), urease, and triple sugar iron (TSI) agar tests. The outcomes were interpreted according to established microbiological criteria and used to confirm the identity of the bacterial species.

**Antibiotic Susceptibility Testing**

The antimicrobial susceptibility of the bacterial isolates was assessed using the Kirby–Bauer disc diffusion method, following CLSI guidelines. Test antibiotics included ciprofloxacin (5 µg), ampicillin (10 µg), erythromycin (15 µg), penicillin (5 µg), and tetracycline (30 µg). Standardized inocula were spread on Mueller–Hinton agar plates using the lawn culture technique, after which antibiotic discs were aseptically placed on the surface. Plates were incubated at 37 °C for 24 hours, and inhibition zone diameters were measured in millimetres. Results were interpreted as Sensitive (S), Intermediate (I), or Resistant (R) according to CLSI criteria.

**Evaluation of Antibacterial Activity of Bee Honey**

The antibacterial activity of bee honey was assessed using the agar well diffusion method against both Gram-positive (*Staphylococcus.aureus and Streptococcus. pyogenes)* and Gram-negative (*Klebsiella. pneumoniae*, *Pseudomonas. aeruginosa*, and *Proteus. mirabilis*) bacterial pathogens.

Fresh bacterial cultures were standardized and inoculated onto Mueller–Hinton Agar plates by the lawn culture method to obtain uniform bacterial growth. Sterile wells of approximately 6 mm diameter were aseptically bored into the agar using a sterile cork borer. Aliquots of the prepared honey dilutions (5%, 10%, and 20% v/v) were dispensed into the respective wells, while control wells containing sterile distilled water were included for comparison.

The inoculated plates were allowed to stand at room temperature for 30 min to facilitate diffusion of the honey samples into the agar medium. The plates were subsequently incubated at 37°C for 24 h. Following incubation, antibacterial activity was evaluated by measuring the diameter of the clear zones of inhibition surrounding each well. The results were recorded in millimetres (mm), and the antibacterial efficacy of the different honey concentrations was compared with that of the selected commercial antibiotics (Mandal MD, Mandal *et al.*, 2011).

**RESULT:**

Table 1 : **Antibiotic sensitivity of bacterial spp**

| Sl | Bacteria | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | Ampicillin (AMP) | Penicillin (P) | Erythromycin(E) | Tetracycline(TE) | Ciprofloxacin(CIP) |
| 1 | *Staphylococcus aureus* | R | R | 12 | 11 | 15 |
| 2 | *Streptococcus pyogenes* | R | R | 13 | 6 | 13 |
| 3 | *Klebsiella pneumoniae* | R | R | R | R | 12 |
| 4 | *Pseudomonas aeruginosa* | R | R | 11 | 7 | 12 |
| 5 | *Proteus mirabilis* | R | R | 14 | 5 | 14 |

Resistant-No inhibition zone

Table 2: **Antibacterial effect of bee honey on different bacterial pathogens:**

| Sl | Organism | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | Bee honey extract in different concentration | | | |
| | | Control | 5% | 10% | 20% |
| 1 | *Staphylococcus aureus* | R | 5 | 13 | 20 |
| 2 | *Streptococcus pyogenes* | R | R | 2 | 10 |
| 3 | *Klebsiella pneumonia* | R | 9 | 13 | 25 |
| 4 | *Pseudomonas aeruginosa* | R | R | R | R |
| 5 | *Proteus mirabilis* | R | 8 | 11 | 21 |

**Table 3:**

**Comparative Evaluation of the Antibacterial Efficacy of Bee Honey and Commercial Antibiotics Against Clinically Relevant Bacterial Pathogens**

| Sl no | Antibiotics and zone of Inhibition | | | | | | Bee honey concentration. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Name | Zone of clearance | | | | | 5% | 10% | 20% |
| | | AMP | P | E | TE | CIP | | | |
| 1 | *Staphylococcus aureus* | R | R | 12 | 11 | 15 | 5 | 13 | 20 |
| 2 | *Streptococcus pyogenes* | R | R | 13 | 6 | 13 | R | 2 | 10 |
| 3 | *Klebsiella pneumonia* | R | R | R | R | 12 | 9 | 13 | 25 |
| 4 | *Pseudomonas aeruginosa* | R | R | 11 | 7 | 12 | R | R | R |
| 5 | *Proteus mirabilis* | R | R | 14 | 5 | 14 | 8 | 11 | 21 |

**Antibiotic sensitivity of bacterial spp**

The antibiotic susceptibility pattern of the tested bacterial isolates revealed that all organisms were resistant to ampicillin and penicillin, showing no zones of inhibition. Ciprofloxacin exhibited the highest antibacterial activity against all isolates, with inhibition zones ranging from 12–15 mm. Erythromycin showed moderate activity against Staphylococcus aureus (12 mm), *Streptococcus pyogenes (*13 mm), *Pseudomonas aeruginosa* (11 mm), and *Proteus mirabilis* (14 mm), while *Klebsiella pneumoniae* was resistant. Tetracycline demonstrated comparatively lower activity, with inhibition zones ranging from 5–11 mm, and *Klebsiella pneumoniae* . remained resistant. Overall, ciprofloxacin was the most effective antibiotic, whereas *K.pneumoniae* exhibited the highest level of antibiotic resistance among the tested pathogens (Table 1).

**Antibacterial effect of bee honey on different bacterial pathogens:**

The antibacterial activity of bee honey varied according to its concentration and the bacterial species tested. No inhibitory effect was observed in the control group. The 20% honey concentration exhibited the highest antibacterial activity, producing inhibition zones of 25 mm against *Klebsiella pneumoniae,* 21 mm against *Proteus mirabilis*, 20 mm against *Staphylococcus aureus*, and 10 mm against *Streptococcus pyogenes.* Moderate antibacterial activity was observed at the 10% concentration, while the 5% concentration showed only limited inhibitory effects. *Pseudomonas aeruginosa* was resistant to all honey concentrations tested and showed no zone of inhibition. Overall, the antibacterial efficacy of bee honey increased with concentration, with the 20% honey extract demonstrating the strongest inhibitory activity against most of the tested bacterial pathogens (Magiorakos AP, Srinivasan A, Carey RB, *et al* .,2012) (Table 2).

**Comparative Evaluation of the Antibacterial Efficacy of Bee Honey and Commercial Antibiotics Against Clinically Relevant Bacterial Pathogens**

The comparative analysis of antibiotic susceptibility and bee honey activity revealed distinct differences in antibacterial efficacy against the tested pathogens. All organisms showed complete resistance to ampicillin and penicillin. Ciprofloxacin exhibited the highest overall antibacterial activity, producing inhibition zones ranging from 12 to 15 mm, followed by erythromycin (11–14 mm) and tetracycline (5–11 mm), while *Klebsiella pneumoniae* remained resistant to multiple antibiotics.

In comparison, bee honey demonstrated a concentration-dependent antibacterial effect. The 20% honey concentration showed the strongest activity, producing larger zones of inhibition than several antibiotics against certain organisms, notably *Klebsiella pneumoniae* (25 mm) and *Proteus mirabilis* (21 mm), and comparable activity against *Staphylococcus aureus* (20 mm). Moderate activity was observed at 10%, whereas the 5% concentration showed limited or no effect against some isolates, including *Pseudomonas aeruginosa*, which remained resistant to both antibiotics and honey.

Overall, while ciprofloxacin was the most consistently effective antibiotic, bee honey—particularly at higher concentrations—demonstrated significant antibacterial potential and in some cases exceed the activity of conventional antibiotics (Nolan VC, Harrison J, Cox JAG.,*et al* 2012) (Table 3).

**DISCUSSION:**

This study investigated the antibacterial activity of bee honey against *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis*. Honey solutions of different concentrations exhibited concentration-dependent inhibition, with 20% honey showing the strongest effect. The antimicrobial properties are attributed to bioactive phytochemicals, including flavonoids, alkaloids, tannins, phenolics, and steroids, which act through mechanisms such as cell wall disruption, inhibition of protein and nucleic acid synthesis, and interference with enzymatic activity. These findings highlight bee honey as a promising natural antimicrobial agent with potential therapeutic relevance as an alternative or adjunct to conventional antibiotics.

**CONCLUSION :**

The present study demonstrates that bee honey possesses considerable antibacterial activity against the selected pathogenic microorganisms, highlighting its potential as a natural antimicrobial agent. The antibacterial effect was found to be concentration-dependent, with higher concentrations exhibiting greater inhibitory activity against bacterial growth. In some cases, the efficacy of bee honey was comparable to that of conventional antibiotics, emphasizing its therapeutic potential.

These findings suggest that bee honey could serve as a promising alternative or complementary treatment for bacterial infections, particularly in light of the growing challenge of antimicrobial resistance and the adverse effects associated with synthetic antibiotics. Owing to its broad-spectrum antibacterial properties and natural origin, bee honey holds significant promise for pharmaceutical and clinical applications. However, further in-depth investigations are required to elucidate its mechanism of action, evaluate its safety and efficacy through clinical studies, and facilitate the development of standardized natural antimicrobial formulations (Kwakman PHS, Zaat SA *et al.,2012)*

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