HSJ

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

Vol.4, Issue. 1

Received: 15 November 2025 Accepted: 05 December 2025 Published: 19 December 2025

MDRI

Research Article

COMPARATIVE EVALUATION OF ANTIBACTERIAL ACTIVITY OF (ACACIA) HONEY AND COMMONLY USED ANTIBIOTICS AGAINST *KLEBSIELLA* SPECIES ISOLATED FROM UTI PATIENTS

Nazia bibi[1] | Faqiaha Wadood[1] | Faiqa Ramzan[1] | Samia ijaz[1] | Rifat bibi[1] | Atta Ur Rehman*[1] | Muhammad Tariq[1] | Sana Ullah[1] | Anila Khan[2] | Hussan Ara Begum[3]

[1]Faculty of Veterinary and Animal Sciences, Gomal University D.I. Khan
[2]Institute of Biological Sciences, Gomal University D.I. Khan
[3]Honeybee Research Lab (HRL), Department of Entomology Faculty of Agriculture Gomal University D.I. Khan

Correspondence
Atta Ur Rehman
Email: arehman@ gu.edu.pk

Citation
Bibi N, Wadood F, Ramzan F, Ijaz S, Bibi R, Rehman AU, Tariq M, Ullah S, Khan A, Begum HA, Comparative evaluation of Antibacterial activity of (acacia) honey and commonly used antibiotics against *klebsiella* species isolated from uti patients, Health Sciences Journal, 30-37

ABSTRACT:

Background: Urinary tract infections (UTIs) caused by *Klebsiella* species present a growing public health challenge because of increasing multidrug resistance.

Objectives: The antibacterial effectiveness of Acacia honey against *Klebsiella Pneumoniae* isolated from UTI patients is assessed in this study in comparison to routinely used drugs.

Methods: Thirty urine samples in all were gathered and subjected to culture, gram stained, and biochemical analysis to identify the bacteria. Antibacterial assay to check the susceptibility of standard antibiotics and efficacy of honey.

Results: Results indicated that Acacia honey exhibited a significantly larger zone of inhibition (20 mm) compared to conventional antibiotics, including ciprofloxacin (10.2 mm), doxycycline (10.1 mm), and no inhibition by ampicillin, aztreonam, and chloramphenicol. These findings suggest that Acacia honey holds promise as an alternative or complementary treatment for multidrug-resistant *Klebsiella* infections, warranting further exploration of its clinical applications.

Conclusion: Future perspectives are recommended that there is a lack of molecular studies as to how honey can exert its antimicrobial activity at a molecular level e.g. disruption of the cell walls, prevention of biofilm formation or interference with bacterial enzymes

Keywords*:*
Acacia honey, *Klebsiella* species, *Klebsiella Pneumoniae*, Urinary tract infections (UTIs), Multidrug resistance (MDR), Antibacterial activity, Disc diffusion method, Minimum Inhibitory Concentration (MIC),

1 | INTRODUCTION

Klebsiella species, particularly *Klebsiella pneumoniae*, remain significant contributors to urinary tract infections and have gained growing attention due to their increasing ability to develop multidrug resistance.[1] These Gram-negative bacteria possess the capacity to colonize various parts of the urinary tract, where they may cause infections ranging from mild cystitis to severe and complicated pyelonephritis. Although they are generally part of the normal gut microbiota in healthy humans, their transition from harmless colonizers to opportunistic pathogens often occurs when they access vulnerable anatomical sites such as the bladder or kidneys. Their rising detection in UTIs worldwide has placed added pressure on healthcare systems, particularly in regions where antibiotic resistance is already a concern. This trend has highlighted the ongoing need for reliable, safe, and effective treatment options capable of addressing both typical and resistant strains of these pathogens.[2]

Beyond the urinary tract, *Klebsiella* species are associated with a wide range of infections that affect multiple systems in the human body. These include pneumonia, liver abscess, bloodstream infections, meningitis, and infections of wounds and soft tissues. They are commonly present in the nose, throat, skin, and intestinal flora and frequently become pathogenic in individuals who have underlying risk factors such as diabetes, chronic illness, prolonged hospitalization, or the use of invasive medical devices. Their remarkable ability to adhere to and colonize urinary tissues, evade normal immune defenses, and produce enzymes and toxins that damage host tissues contributes to their success as uropathogens. The thick polysaccharide capsule that surrounds these bacteria plays a central role in virulence. It not only provides protection from phagocytosis but also enhances survival in harsh environments and promotes transmission within both community and hospital settings.[3] the combination of adherence structures, protective capsules, and adaptive mechanisms makes *Klebsiella* a challenging pathogen to control. One of the most pressing concerns in the management of *Klebsiella* infections is the rapid rise of antibiotic resistance. Over the past decade, resistance patterns have shifted considerably, with an increasing number of isolates producing extended-spectrum beta-lactamases (ESBLs), which render many commonly used beta-lactam antibiotics ineffective.[4] This resistance severely restricts treatment choices and increases the risk of therapeutic failure, longer hospital stays, and higher healthcare costs. Widespread misuse and overuse of antibiotics in both human medicine and agriculture have accelerated this trend, allowing resistant uropathogenic strains to spread more readily.[5] These resistant organisms not only persist within the host but also carry a diverse set of virulence factors—including adhesins, iron-acquisition systems, and capsule polysaccharides—that further enhance their ability to survive, grow, and cause disease.[6] The combination of multidrug resistance and potent virulence mechanisms makes the control of *Klebsiella* infections a significant challenge for clinicians worldwide.

The growing concerns surrounding antibiotic resistance have prompted renewed interest in natural antimicrobial agents, particularly those with long histories of use and minimal side effects. Honey has been used for centuries as a traditional remedy for infections due to its natural antibacterial, antioxidant, and wound-healing properties. Among various types of honey, Acacia honey has gained scientific attention for its high purity, mild flavor, and strong antimicrobial potential. Studies have shown that honey's antibacterial activity may be attributed to several factors, including its acidic pH, high osmolarity, hydrogen peroxide content, and the presence of bioactive compounds such as flavonoids and phenolic acids.[7] These properties may offer effects against both sensitive and resistant strains of bacteria, including those that cause UTIs. Evaluating the antibacterial properties of honey alongside commonly used antibiotics may help identify whether honey can serve as an alternative or supportive therapeutic option, especially in cases where antibiotic resistance limits treatment choices. The present study aims to isolate and identify *Klebsiella* species from patients with urinary tract infections and to assess the antibacterial activity of honey against these isolates using in vitro susceptibility techniques. In addition, it seeks to compare the antibacterial effectiveness of honey with that of commonly used antibiotics. This comparison will help determine whether honey can serve as a supportive or alternative approach for managing UTIs, particularly in the context of rising antibiotic resistance among *Klebsiella* strains.[8]

2 | MATERIAL AND METHODS

The study was conducted in Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan, where urine samples were collected from patients attending the District Headquarters Hospital. All microbiological analysis was performed in the Microbiology Laboratory at Gomal University. The geographical setting along the west bank of the Indus River and the city's elevation of about 175 meters provided a suitable environment and access to a diverse clinical population for sampling. The research spanned three months, from August to October 2024, ensuring a consistent timeframe for sample collection and laboratory work. A total of 30 patients who met the inclusion criteria were selected consecutively, balancing practical feasibility and the requirements for reliable laboratory evaluation. Ethical approval for the study was granted by the Ethical Review Board of Gomal University (Ref. No. 30/ERB/GU), and written informed consent was obtained from all participants.

2.1 | Sample Collection and processing

Patients clinically diagnosed with urinary tract infections were invited to take part in the study. Midstream urine samples were collected aseptically in sterile containers and promptly transported to the laboratory for the isolation and identification of *Klebsiella pneumoniae*. The study included adults aged 18 years or older with clinical signs of UTI. Individuals who had recently taken antibiotics, those with catheter-associated urinary infections, or those experiencing infections from other anatomical sites were excluded to maintain the consistency and reliability of bacterial isolates. Sample processing involved culturing on CLED agar, MacConkey agar, performing Gram staining, and preparing antibiotic discs. Direct microscopic examination was carried out by centrifuging urine samples at 2500 rpm for four minutes and examining the sediment under the microscope. The presence of pus cells supported the diagnosis of UTI. Urine samples were inoculated onto CLED agar to support the growth of urinary pathogens, differentiate lactose

fermenters, and prevent swarming by *Proteus* species. Suspicious colonies were transferred to MacConkey agar, a selective medium for Gram negative organisms, particularly members of the Enterobacteriaceae family.
Mucoid colonies presumed to be *Klebsiella* were Gram-stained to confirm their morphology. Slides were heat-fixed and sequentially treated with crystal violet, iodine, acetone, and safranin before being examined under oil immersion. Gram negative rods supported the presumptive identification of *Klebsiella*. Biochemical characterization included the catalase test, oxidase test, and API 10E identification. The catalase test was confirmed by bubbling after hydrogen peroxide application. The oxidase test was performed using reagent-soaked filter paper, with *Klebsiella* showing no color change. The API 10E system was used by suspending isolated colonies in sterile water, inoculating the strip, incubating it at 37°C for 18–24 hours, and comparing color changes with the reference database for species confirmation.

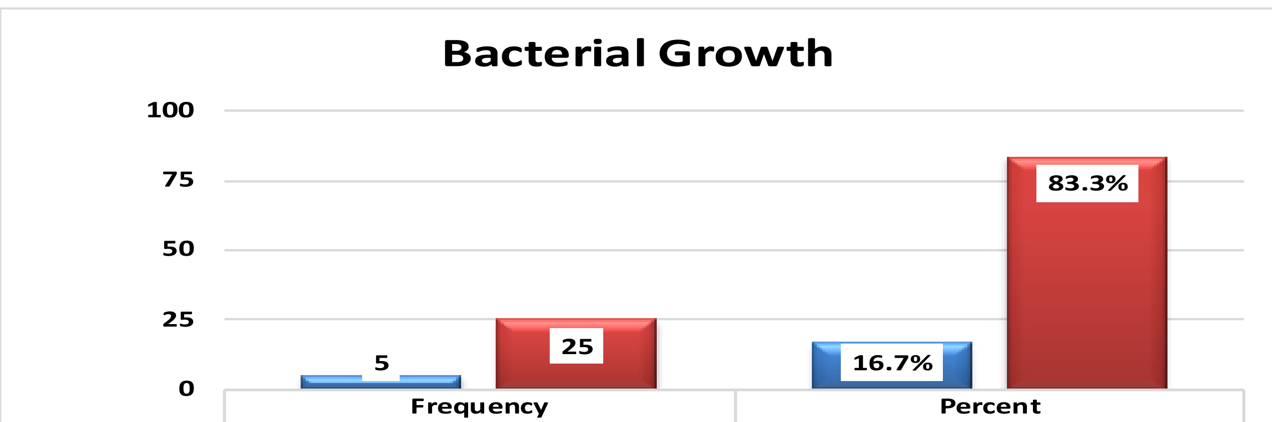
Acacia honey samples were collected from two natural honey centers in Dera Ismail Khan. Each sample was tested in its undiluted form and in freshly prepared dilutions of 1:2, 1:4, 1:6, and 1:8 to assess concentration-dependent antibacterial activity. The activity of honey was compared with standard antibiotics commonly prescribed for UTIs, including ciprofloxacin, ampicillin, doxycycline, aztreonam, and chloramphenicol. These antibiotics were tested using their standard disc concentrations. Antibacterial susceptibility testing was conducted using the disk diffusion method on nutrient agar. Bacterial suspensions were adjusted to a 0.5 McFarland standard and evenly swabbed onto agar plates. After drying, honey droplets and antibiotic discs were applied. The plates were incubated at 37°C for 24 hours, and inhibition zones were measured in millimeters to assess antibacterial effects. All observations were compiled, tabulated, and analyzed using appropriate descriptive and statistical methods to compare the antibacterial activity of honey and standard antibiotics.

3 | RESULTS AND DISCUSSION

The results of the current study depicted included isolating *Klebsiella* species from urine samples, purifying them on selective media, identifying the isolates using API 10S kits, and evaluating the antibacterial activity of antibiotics and honey using disc diffusion, MIC (Minimum Inhibitory Concentration), and MBC (Minimum Bactericidal Concentration) techniques. The results are logically constructed to accomplish the objectives of the study.

3.1 | Isolation of Bacterial Pathogens on CLED Agar

Urinary pathogens were detected in 5 samples (16.6%) of the 30 urine samples that had bacterial growth. Lactose fermentation, a trait of *Klebsiella* species, was evident from the colonies' yellow appearance on CLED Agar. A low level of bacterial contamination was indicated by the five (16.7%) positive results out of 30 samples that were tested for bacterial growth. Most of the samples analyzed, however, did not reveal bacterial growth, as seen by the 25 samples (83.3%) that tested negative. This implies that bacterial growth in the samples examined is not very widespread.



	Frequency	Percent
Positive	5	16.7
Negative	25	83.3

Figure 1 Bacterial Growth chart

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

3.2 | Purification on MacConkey Agar

To purify the bacterial colonies and verify their lactose fermentation status, the five isolates that tested positive on CLED Agar were cultivated further on MacConkey Agar. A selective medium called MacConkey Agar is used to separate lactose fermenters from non-lactose fermenters and to extract Gram-negative bacteria, especially those belonging to the *Enterobacteriaceae* family. It was confirmed that all five isolates were lactose-fermenting Gram-negative bacteria when they displayed pink colonies on MacConkey Agar. The main characteristic of *Klebsiella Pneumoniae* is lactose fermentation, which is shown by the pink coloring.

3.3 | Gram Staining of Isolates

Gram staining was used to ascertain the morphological traits of each of the 5 positive isolates. A crucial technique for distinguishing between Gram-positive and Gram-negative bacteria as well as for examining bacterial morphology (rod-shaped, cocci, etc.) is Gram staining.
The isolates showed Gram-negative rods, confirming that the bacteria belong to the *Enterobacteriaceae* family, which includes *Klebsiella* species. The identification of the common urinary pathogen *Klebsiella Pneumoniae* is consistent with the observation of Gram-negative rods.

3.4 | Identification Using API 10S Kit

The bacterial species was biochemically confirmed using the API 10S Kit. Based on their metabolic traits and enzymatic activity, the kit offers a series of biochemical tests to identify Gram-negative bacteria.

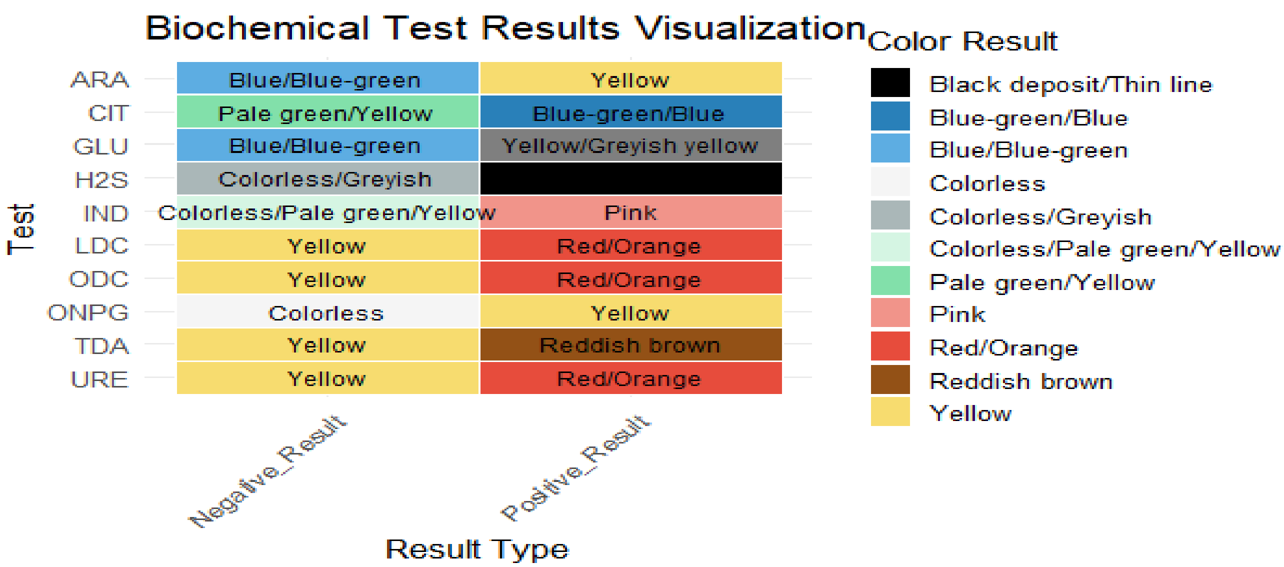


Figure 2 Biochemical Test Results Visualization of *Klebseilla Pneumuniae*

All five positive *Klebsiella Pneumoniae* isolates from the 30 urine samples were identified using the API 10S Kit. The test results demonstrated that all five isolates had positive results for the following: URE (Red/Orange) for urease production; CIT (Blue-green/Blue) for citrate utilization; H2S (Black deposit/Thin line) for hydrogen sulfide production; ONPG (Yellow), which indicates lactose fermentation; GLU (Yellow/Greyish yellow) for glucose fermentation; and ARA (Yellow) for arabinose fermentation. However, the isolates had negative findings for TDA (reddish brown), which indicated no production of tryptophan deaminase, IND (pink), which indicated no formation of indole, and LDC (red/orange), which indicated no lysine decarboxylation. The API code that corresponded to *Klebsiella Pneumoniae* further validated the biochemical pattern seen in the test, which was in line with the traits of this species. Gram Staining, MacConkey Agar, and CLED Agar findings that also identified the isolates as *Klebsiella Pneumoniae* are consistent with our results. The results gathered from the API 10S Kit demonstrated that *Klebsiella Pneumoniae* was the cause of the urinary tract infections in the patients who were sampled and confirmed the bacterium's identity.

Table 1 Biochemical characterization list of *Klebseilla Pneumuniae*

Test	Key Substrate	Enzyme or Reaction Detected	Negative Appearance	Positive Appearance
ONPG	2-Nitrophenyl-β-D-galactopyranoside	Activity of β-galactosidase	No color change	Yellow color develops
GLU	D-Glucose	Glucose fermentation or oxidation	Blue or blue-green	Yellow or grey yellow
ARA	L-Arabinose	Arabinose fermentation or oxidation	Blue or blue green	Yellow
LDC	L-Lysine	Lysine decarboxylase activity	Yellow	Red or orange
ODC	L-Ornithine	Ornithine decarboxylase activity	Yellow	Red or orange
CIT	Trisodium citrate	Citrate utilization	Pale green or yellow	Blue-green or blue
H_2S	Sodium thiosulfate	Hydrogen sulfide production	No blackening	Black precipitate or thin black line
URE	Urea	Urease activity	Yellow	Red or orange
TDA	L-Tryptophan	Tryptophan deaminase activity	Yellow	Brownish-red coloration
IND	L-Tryptophan	Indole production	Colorless, pale green, or yellow	Pink ring or layer

3.5 | Antimicrobial Efficacy of Honey and Antibiotics

The disc diffusion method was used to assess honey's antibacterial effectiveness on nutrient agar. The honey sample's zone of inhibition was assessed and compared to the effectiveness of five widely used antibiotics. These were the antibiotics that were tested.

Table 2 Comparison of Zone of inhibition in millimeter of antibiotics and honey

Antimicrobial Agent	Abbreviation	Disc Content	Zone of Inhibition
Honey	-	20 µL	20 mm
Ciprofloxacin	CIP	5 µg	10.2 mm
Doxycycline	DOX	20 µg	10.1 mm
Ampicillin	AMP	10 µg	No Inhibition
Aztreonam	ATM	30 µg	No Inhibition
Chloramphenicol	C	30 µg	No Inhibition

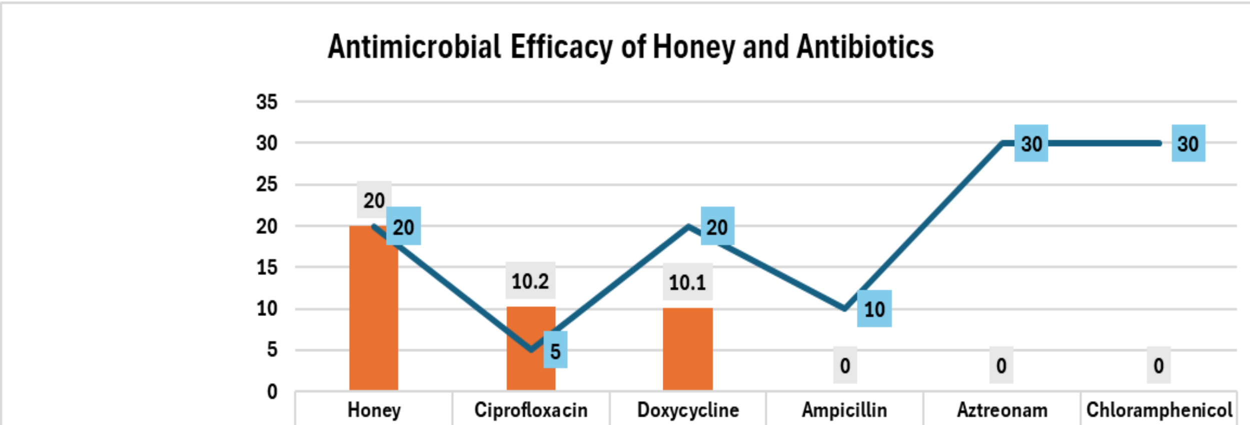
According to the data, honey had a 20 mm zone of inhibition, which was significantly larger than any of the tested antibiotics, indicating its potent antibacterial activity. The antibiotic results were as follows, Ciprofloxacin (Cip): 10.2 mm zone of inhibition, indicating moderate susceptibility. Doxycycline (DOX): 10.1 mm zone of inhibition, showing moderate antibacterial activity. Ampicillin (AMP), Aztreonam (ATM), and Chloramphenicol (C): All three antibiotics showed no inhibition, indicating resistance by the *Klebsiella Pneumoniae* isolates.

Table 3 The Distribution of a Dataset Related to Measurements in Millimeters.

Metric	Value
Minimum	0.00 mm
1st Quartile	0.00 mm
Median	5.05 mm
Mean	6.72 mm
3rd Quartile	10.18 mm
Maximum	20.00 mm
SD	8.19 mm

$p = 0.2134 > 0.05 \rightarrow$ Not statistically significant

According to the results, honey may be more efficient than the tested antibiotics in addition to having antibacterial action, particularly when *Klebsiella Pneumoniae* isolates exhibit resistance to popular antibiotics like ampicillin, aztreonam, and chloramphenicol. The potential use of honey as an alternative or supplemental treatment for *Klebsiella Pneumoniae* infections has been shown by its ability to effectively limit bacterial growth. This outcome also emphasizes how crucial it is to consider natural antimicrobials like honey, especially in view of the growing antibiotic resistance. To improve treatment results, more research could examine the synergistic effects of mixing honey with antibiotics.



	Honey	Ciprofloxacin	Doxycycline	Ampicillin	Aztreonam	Chloramphenicol
Zone of Inhibition (mm)	20	10.2	10.1	0	0	0
Disc Content (ug)	20	5	20	10	30	30

Figure 3 Antimicrobial efficacy of honey and antibiotics

4 | DISCUSSION

In this study, *Klebsiella* species were isolated from urine samples, the bacterial isolates were purified and identified, and the antibacterial activity of honey was evaluated in comparison to commonly used antibiotics using a variety of techniques, for example, disc diffusion. Given the rise in antibiotic resistance, the results of this study advance our knowledge of *Klebsiella* infections and the potential of honey as a substitute antimicrobial agent.[9] The isolates also demonstrated pronounced resistance to most used antibiotics—ampicillin, aztreonam, and chloramphenicol—thus indicating the increasing trend of antibiotic resistance (AMR). Similar resistance patterns have been observed in other parts of the world including Pakistan, making it extremely necessary to develop alternative therapies to address the issue.[10]

Using CLED (Cystine Lactose Electrolyte Deficient) agar, *Klebsiella* species were successfully isolated from urine samples; 16.6% (5/30) of the samples displayed bacterial growth. The results of earlier research, which regularly linked *Klebsiella* species to urinary tract infections (UTIs), are in line with this. Since *Klebsiella* species are known to ferment lactose, the lactose fermentation property, which produced yellow colonies on CLED agar, was essential for identifying them. *Klebsiella* species identification was further supported by further purification on MacConkey agar, which verified the isolates' status as lactose fermenters.[11] The isolates were shown to be Gram-negative rods that were compatible with *Klebsiella pneumoniae* based on their morphological and biochemical traits, which were validated by Gram staining and the API 10S kit. These results are consistent with earlier publications and other research that has shown how common *Klebsiella pneumoniae* is in urinary tract infections.[12]

The most striking finding was that Manuka honey UMF+15 could suppress carbapenem-resistant Enterobacteriaceae (including *Klebsiella pneumoniae*) at low concentrations (15–18%), resulting in inhibition zones of approximately 2–14 mm at different concentrations. Although the approaches differ, the similarity lies in the tendency of honey to maintain antimicrobial effects where antibiotics do not.[13] Honey was more active against *Klebsiella pneumoniae* with a 20 mm zone of inhibition, compared to ciprofloxacin (10.2 mm) and doxycycline (10.1 mm) in this study, while ampicillin, aztreonam, and chloramphenicol did not show any effect. This agrees with literature encapsulating extensive evidence that honey frequently performs better than conventional antibiotics, even against multidrug-resistant (MDR) strains.[14]

This in vitro study verified that stingless bee honey and *Apis mellifera* honeys (at 50% v/v) produced zones of inhibition of approximately 22–27 mm against clinical MDR strains of *Klebsiella pneumoniae*, as well as low MIC values (~6.25–12.25%).[15]

Honey's antibacterial effectiveness was assessed using the disc diffusion method and contrasted with that of five widely used antibiotics: ampicillin, aztreonam, doxycycline, ciprofloxacin, and chloramphenicol. In contrast to all antibiotics studied, honey showed an impressive 20 mm zone of inhibition. The findings align with earlier research demonstrating honey's broad-spectrum antibacterial activity, particularly against urinary pathogens such as *Klebsiella* species. Given its wide zone of inhibition, honey may be a useful supplement or substitute treatment for *Klebsiella* infections, particularly when antibiotic resistance is common.[16] Among the investigated antibiotics, doxycycline and ciprofloxacin showed modest antibacterial activity, with inhibition zones of 10.1 mm and 10.2 mm, respectively. Conversely, the *Klebsiella* isolates were resistant to ampicillin, aztreonam, and chloramphenicol. This finding aligns with reports that hospital-associated infections caused by *Klebsiella* species are becoming increasingly resistant to antibiotics.[17]

Alternative treatments are necessary because *Klebsiella pneumoniae* resistance to multiple drugs poses a serious problem in clinical settings. As multidrug-resistant strains of *Klebsiella* have evolved, concern has increased globally. This investigation demonstrated that *Klebsiella pneumoniae* is resistant to commonly used antibiotics such as ampicillin, aztreonam, and chloramphenicol.[18] The superior effectiveness of honey in inhibiting bacterial growth, compared with the inability of these antibiotics, highlights the urgent need for alternative antimicrobial agents. Honey's ability to inhibit bacterial growth, particularly in strains resistant to conventional antibiotics, makes it a promising treatment option for UTIs caused by drug-resistant pathogens.

Current research supports the potency of natural honeys against *Klebsiella pneumoniae* with respect to antibacterial and antibiofilm activities. For example, Iranian thyme and citrus honeys collected during 2023–2024 showed significant inhibitory effects against *Klebsiella pneumoniae* in both planktonic and biofilm states, supporting their role as safe complementary treatments in infectious diseases.[19] For clinical practice, the findings of this study have important implications. The growing resistance of *Klebsiella pneumoniae* to commonly used antibiotics such as ampicillin and aztreonam necessitates alternative or adjunct therapies. Given its strong antibacterial properties, honey may serve as a viable alternative or complementary treatment for *Klebsiella*-associated UTIs, particularly in cases of antibiotic resistance. Evidence also suggests that honey can act synergistically with antibiotics, supporting its use in combination therapy.[16]

Future studies should focus on determining optimal honey concentrations required to inhibit bacterial growth and evaluating potential synergistic effects with antibiotics. Clinical trials are also needed to assess the safety and efficacy of honey in treating UTIs and other infections caused by resistant bacteria. Further investigation into the molecular mechanisms underlying honey's antibacterial activity may provide valuable insights into its interaction with bacterial cells and its role in bacterial suppression.

5 | CONCLUSION

The study aimed to isolate *Klebsiella Pneumoniae* from urine samples, assess its antimicrobial resistance, and evaluate the antibacterial potential of honey compared to common antibiotics. The results showed that *Klebsiella Pneumoniae* is a common pathogen in urinary tract infections (UTIs), with 16.6% of samples showing growth. Honey's antimicrobial efficacy was evaluated, showing a large zone of inhibition (20 mm) surpassing the activity of commonly used antibiotics like Ciprofloxacin and Doxycycline. This suggests that honey may serve as a potent alternative or adjunct therapy, particularly in regions where antibiotic resistance is rising.

The implications of this study are significant for clinical practice, as the increasing resistance of *Klebsiella Pneumoniae* to commonly used antibiotics necessitates alternative treatment strategies. Honey's antimicrobial properties provide a promising solution, particularly for UTIs caused by antibiotic-resistant strains. The potential synergistic effects of honey when combined with antibiotics could open new avenues for overcoming resistance. Further research is needed to identify optimal concentrations of honey, understand its molecular mechanisms, and conduct clinical trials to establish its safety and efficacy in treating infections.

6 | LIMITATIONS

This study was limited by a small sample size and an in vitro design, which may not fully reflect in vivo conditions. Only Acacia honey was tested, so results cannot be generalized to other honey types. The antibacterial assessment relied mainly on the disc diffusion method, without MIC or MBC determination. Mechanisms of action, molecular characterization of resistance, and clinical safety or synergistic effects with antibiotics were not evaluated.

Conflict of Interest statement: All authors declare no conflict of interest
Data Availability Statement: Data was available from the primary author and will be provided on special request
Authors' Contribution: All authors equally contributed to writing, reviewing and finalizing the draft
Funding: Not Applicable
Informed consent: Not Applicable
Ethical Approval: Not Applicable
Acknowledgement: We would like to express profound gratitude to every participant who shown their consent and cooperation for their data of this study.

REFERENCES

1. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol*. 2005;43(11):5721–32.
2. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner ACR, Yu WH, Lakshmanan A, Wade WG. The human oral microbiome. *J Bacteriol*. 2010;192(19):5002–17.
3. Marsh PD, Zaura E. Dental biofilm: ecological interactions in health and disease. *J Clin Periodontol*. 2017;44(Suppl 18):S12–22.
4. Kilian M, Chapple ILC, Hannig M, Marsh PD, Meuric V, Pedersen AML, Tonetti MS, Wade WG, Zaura E. The oral microbiome—an update for oral healthcare professionals. *Br Dent J*. 2016;221(10):657–66.
5. Rajasekaran JJ. Oral microbiome: a review of its impact on oral and systemic health. *Microorganisms*. 2024;12(9):1797.
6. Socransky SS, Haffajee AD. The microbial etiology of destructive periodontal disease: current concepts. *J Periodontol*. 2005;76(10):206–17.
7. Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: more and more importance in oral cavity and systemic diseases. *Front Cell Infect Microbiol*. 2018;8:54.
8. Arweiler N, Netuschil L. The oral microbiota: a dynamic interplay with the host. *Periodontol 2000*. 2016;70(1):21–36.
9. Diaz PI, Hoare A, Dongari-Bagtzoglou A. A dynamic view of the oral microbiome in health and disease. *J Dent Res*. 2017;96(12):1349–57.
10. Belstrøm D, Holmstrup P, Nielsen CH, Kirkby N, Twetman S, Heitmann BL, Klepac-Ceraj V, Paster BJ, Fiehn NE. Salivary microbiota in dental health and disease. *J Oral Microbiol*. 2014;6:26180.
11. Kolenbrander PE, Palmer RJ, Periasamy S, Jakubovics NS. Oral multispecies biofilm development and interactions. *Trends Microbiol*. 2010;18(2):71–8.
12. Fejerskov O, Nyvad B, Kidd E. *Dental Caries: The Disease and Its Clinical Management*. 3rd ed. Oxford: Wiley-Blackwell; 2015.
13. Genco RJ, Van Dyke TE. Prevention: reducing the risk of cardiovascular disease in patients with periodontitis. *Nat Rev Cardiol*. 2010;7(9):479–80.
14. Fitzsimonds RM, Lea R. Antibiotic resistance in oral streptococci. *Infect Drug Resist*. 2019;12:3003–12.
15. Huang X, Browne K. Oral microbiome and childhood caries: a systematic review. *Pediatr Dent*. 2017;39(5):129–39.
16. Peters BM, Jabra-Rizk MA, O'May GA, Costerton JW, Shirtliff ME. Biofilm formation and antimicrobial resistance in oral microbes. *J Dent Res*. 2012;91(8):692–700.
17. Woelber JP, Breidenbach A, Al-Ahmad A. On the pathogenicity of the oral biofilm: a critical review. *Nutrients*. 2022;14(5):1037.
18. Wade WG. Characterisation of the human oral microbiome. *Pharmacol Res*. 2013;69(1):137–43.
19. Willis JR, Gabaldón T. The human oral microbiome in health and disease. *Microorganisms*. 2020;8(2):308.