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MANUKA HONEY AS A POTENTIAL TREATMENT FOR MCR-1 HARBORING E. COLI AND KLEBSIELLA ISOLATES FROM URINARY TRACT INFECTIONS

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Abstract

Background and Rationale: *The effective treatment of urinary tract infections (UTIs) is increasingly threatened by multidrug-resistant (MDR) bacteria such as Escherichia coli and Klebsiella pneumoniae. A key factor in this trend is the worldwide spread of the mcr-1 gene, which is carried on plasmids and provides resistance to colistin, an antibiotic of last resort. This dire situation necessitates the search for alternative antimicrobial agents. Manuka honey, with its well-documented broad-spectrum antibacterial activity, emerges as a viable option for evaluation.*

Objective: *The aim of this in vitro study was to determine the antibacterial effectiveness of Manuka honey against clinical UTI isolates of E. coli and K. pneumoniae that possess the mcr-1 gene.*

Methods: *One hundred clinical MDR isolates (60 E. coli and 40 K. pneumoniae) were used. The presence of the mcr-1 gene in all isolates was verified by PCR. Initial antibiotic susceptibility patterns were established using the Kirby-Bauer disc diffusion method. The primary evaluation of Manuka honey (UMF 15+) was conducted using an agar well diffusion assay, testing concentrations of 5%, 10%, 20%, and 40% (v/v). Furthermore, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were precisely determined through microbroth dilution assays.*

Results: *All isolates were confirmed as mcr-1 positive and exhibited extensive drug resistance, including 100% resistance to cefepime, cefotaxime, and colistin. Manuka honey demonstrated significant, dose-dependent antibacterial activity. At 40% concentration, the mean zones of inhibition were 18.2 ± 0.8 mm for E. coli and 17.5 ± 0.6 mm for K. pneumoniae. The MIC values were 18% (v/v) for E. coli and 22% for K. pneumoniae, with corresponding MBC values of 22% and 25%. No statistically significant difference in susceptibility was observed between the two species (p > 0.05).*

Conclusion: *Manuka honey exhibits potent in vitro bactericidal activity against colistin-resistant, MCR-1 producing E. coli and K. pneumoniae. These findings underscore its potential as a viable alternative or adjunct therapy for combating pan-drug-resistant UTIs, meriting further clinical investigation.*

Keywords: *COVID-19, Diabetes Mellitus, Hypertension, Knowledge, attitude and practice (KAP), Prevention*

INTRODUCTION

Urinary tract infections (UTIs) caused by MDR bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* pose a significant health threat globally, particularly due to the emergence of resistance to common antibiotics (1-3). The World Health Organization (WHO) has classified these pathogens as high-priority organisms due to their association with serious infections and hospital-acquired diseases (3). Antibiotic resistance, particularly in strains harboring the MCR-1 gene, a plasmid-mediated colistin resistance gene, has further complicated treatment options and contributed to the global healthcare burden. These resistant strains pose a significant threat by increasing the risk of treatment failure, resulting in longer hospital stays, higher healthcare costs, and, more importantly, higher mortality rates (4-7). The rapid spread of such resistance highlights the urgent need for alternative treatment strategies to combat these increasingly resilient pathogens.

In recent years, alternative treatments, particularly natural remedies like Manuka honey, have gained attention for their potential antimicrobial properties. Manuka honey, produced from the nectar of the *Leptospermum scoparium* plant, has been well-documented for its antimicrobial efficacy against a wide range of bacteria, including both Gram-positive and Gram-negative pathogens (6). This honey's antibacterial activity is attributed to its unique compounds, particularly methylglyoxal (MGO), which has been shown to exert bactericidal effects by disrupting bacterial cell division, inhibiting biofilm formation, and reducing bacterial adhesion to host cells (7, 8). Furthermore, Manuka honey has demonstrated effectiveness against multidrug-resistant strains, offering a promising alternative to traditional antibiotics, particularly in the face of rising resistance (9-11).

The role of Manuka honey in managing UTIs, especially those caused by MCR-1 positive bacteria has not been extensively studied. In response to the growing challenge of colistin resistance among UTI pathogens, this research assesses the antimicrobial potential of Manuka honey. Specifically, we aim to determine its activity against MDR *E. coli* and *Klebsiella pneumoniae* isolates harboring the MCR-1 gene. The findings from this investigation could position Manuka honey as a promising alternative or adjunctive therapeutic strategy for resistant UTIs. Previous studies have highlighted the potential of Manuka honey in combating a variety of resistant pathogens, including its ability to inhibit biofilm formation in *Staphylococcus aureus*. Additionally, it has shown promise as an effective wound dressing material for chronic, non-healing, discharging wounds (12, 13). Beyond its antibacterial properties, Manuka honey also exhibits antifungal, antiviral, and antidiabetic activities, further expanding its therapeutic potential (14-17). In this study, we focus on evaluating Manuka honey's antibacterial activity against MCR-1 positive isolates from E. coli and Klebsiella to determine its potential as a therapeutic agent in the treatment of resistant UTIs.

METHODOLOGY

ETHICAL CONSIDERATIONS AND ISOLATE COLLECTION

The research was conducted with ethical approval from Khyber Medical University (Ref: Dir/KMU-EB/PR/000884) and involved written informed consent from guardians. A set of 100 MDR *E. coli* and *K. pneumoniae* isolates was procured from UTI patients at PIMS, Islamabad. Patient confidentiality was maintained through anonymous sample processing. The sample size of 100 clinical isolates was selected to provide a robust panel of contemporary, colistin-resistant MDR pathogens, consistent with similar *in vitro* studies to ensure statistically reliable analysis.

MICROBIOLOGICAL PROCEDURES

Initial isolation was performed by inoculating urine samples onto MacConkey agar (Oxoid), Cystine Lactose Electrolyte Deficient (CLED) agar (Oxoid), chocolate agar (prepared from GC agar base with 5% defibrinated horse blood), and 5% sheep blood agar (Oxoid). All culture media were prepared according to the manufacturers' instructions, sterilized by autoclaving at 121°C for 15 minutes where required, and poured into sterile Petri dishes. Inoculated plates were incubated at 37°C for 18-24 hours under aerobic conditions, with chocolate agar plates incubated in a 5% CO2 atmosphere. Species-level identification was conducted through an assessment of colony morphology, Gram stain characteristics, and biochemical tests (API-10S system, bioMérieux). The isolates, chosen for their multidrug-resistant profiles and potential MCR-1 gene presence, were then stored at -80°C in glycerol stock.

ANTIBIOTIC AND MOLECULAR PROFILING

The MDR status was confirmed by antibiotic susceptibility testing against a panel of eight drugs using the Kirby-Bauer disc diffusion method, interpreted per CLSI standards (18). For genetic analysis, plasmid DNA was extracted from colistin-resistant isolates using the alkaline lysis technique. The integrity and concentration of the extracted DNA were subsequently confirmed by spectrophotometry and agarose gel electrophoresis.

PCR ANALYSIS OF mcr-1 GENE

The presence of the mcr-1 gene was detected using PCR amplification. The primers used for PCR amplification of the mcr-1 gene are listed in Table 1. The forward primer (MCR1-F) sequence is CGGTCAGTCCGTTTGTTC, while the reverse primer (MCR1-R) sequence is CTTGGTCGGTCTGTAGGG, producing an amplicon of 309 base pairs. The amplification reaction was performed at an annealing temperature of 52°C. These primers were adapted from the study by Liu et al (19).

PREPARATION OF MANUKA HONEY

Commercially sourced Manuka honey with a UMF 15+ rating was used. For antibacterial testing, the honey was diluted in sterile saline to create working solutions of 5%, 10%, 20%, and 40% (v/v).

ANTIBACTERIAL ASSAYS

The inhibitory effect of the honey was first quantified using the agar well diffusion method. Mueller-Hinton agar plates were seeded with test organisms adjusted to the 0.5 McFarland standard. Wells were bored into the agar and filled with the various honey dilutions. The diameter of the growth inhibition zones around each well was measured after 24 hours of incubation at 37°C.

For a more precise quantitative assessment, a microbroth dilution assay was conducted to establish the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Bacterial inocula were standardized to 1 × 10^5 CFU/mL in a 96-well plate containing serially diluted honey. The MIC was identified as the lowest honey concentration that inhibited visual growth after overnight incubation. Subsequently, samples from non-growing wells were plated on nutrient agar to determine the MBC, defined as the lowest concentration that killed 99.9% of the initial inoculum. Both assays were consistently performed in triplicate to ensure result reliability.

DATA ANALYSIS

All data were analyzed using IBM SPSS Statistics, Version 25.0. Descriptive statistics were presented as mean ± standard deviation (SD) for continuous variables (e.g., zone of inhibition diameters) and as percentages for categorical variables (e.g., antibiotic resistance rates). The normality of the data distribution was confirmed using the Shapiro-Wilk test. Comparisons of the zones of inhibition, MIC, and MBC values between the two bacterial species (E. coli and K. pneumoniae) were performed using an independent samples t-test. A one-way analysis of variance (ANOVA) was used to compare the antibacterial activity across different honey concentrations, followed by a post-hoc Tukey's test for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant for all tests.

RESULTS

ANTIBIOTIC SUSCEPTIBILITY AND MOLECULAR CONFIRMATION OF MCR-1 ISOLATES

All one hundred clinical isolates of *E. coli* and *K. pneumoniae* selected for this study were confirmed to be multidrug-resistant (MDR), displaying resistance to three or more classes of antibiotics. All bacterial isolates demonstrated phenotypic resistance to colistin. To confirm the genetic mechanism behind this resistance, PCR and sequencing were performed. These molecular analyses successfully detected the plasmid-mediated mcr-1 gene in the entire collection of 100 isolates, confirming it as the source of the resistance, a finding summarized in Fig. 1.

The antibiotic susceptibility profiling, as determined by the Kirby-Bauer disc diffusion method, revealed an alarming resistance profile. All mcr-1-positive isolates exhibited significant resistance to the tested antibiotics. The resistance rates were as follows: aztreonam (98%), ciprofloxacin (97%), levofloxacin (96%), cefepime (100%), cefotaxime (100%), and piperacillin/tazobactam (95%). Notably, a high level of resistance was also observed against imipenem (42%), while amikacin remained moderately effective with a resistance rate of 28%. As expected due to the presence of the mcr-1 gene, all isolates were resistant to colistin, leaving few effective therapeutic options. Antibiotic susceptibility pattern of both bacteria are listed in Table I.

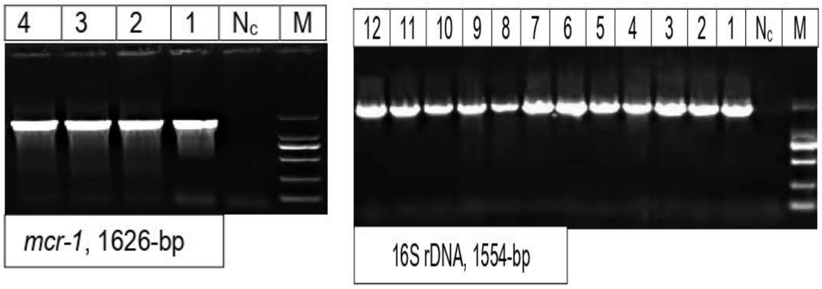

Fig. 1. This figure shows the PCR amplification results for the *mcr-1* gene and 16S rDNA in *E. coli* and *Klebsiella pneumoniae* isolates. The band at 1626-bp indicates the presence of the *mcr-1* gene, confirming colistin resistance. The band at 1554-bp represents the 16S rDNA gene, serving as an internal control to verify the presence of bacterial DNA in all samples

ANTIBACTERIAL EFFICACY OF MANUKA HONEY AGAINST RESISTANT PATHOGENS

Evaluation via the agar well diffusion method revealed substantial antibacterial effects of Manuka honey against all tested mcr-1 positive isolates, with a clear correlation between concentration and inhibitory response. The measured zones of inhibition expanded consistently with increasing honey concentration. For *E. coli* isolates, the mean inhibition zone reached 18.2 ± 0.8 mm at the maximum concentration of 40% (v/v), while *K. pneumoniae* exhibited a similar inhibition zone of 17.5 ± 0.6 mm. Notably, significant antimicrobial activity persisted at the intermediate 20% (v/v) concentration, producing mean zones of 12.1 ± 0.5 mm and 11.8 ± 0.7 mm for *E. coli* and *K. pneumoniae*, respectively.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Manuka honey were determined using the microbroth dilution assay. The results indicated that Manuka honey was effective at inhibiting and killing the colistin-resistant pathogens. The MIC values for Manuka honey against mcr-1 positive *E. coli* and *K. pneumoniae* were 18% v/v and 22% v/v, respectively. The MBC values, confirming the bactericidal nature of the honey, were found to be 22% v/v for *E. coli* and 25% v/v for *K. pneumoniae*. There was no statistically significant difference in the susceptibility between the two bacterial species ($p > 0.05$). The MIC and MBC of different tested concentrations of manuka honey against *E. coli* and *K. pneumoniae* isolates are presented in Table II.

Table I. Antibiotic resistance profile of MCR-1 positive *E. coli* and *K. pneumoniae* isolates (n=100) from urinary tract infections

Antibiotic class	Antibiotic (Abbreviation)	*E. coli* (n=60) Resistant (%)	*K. pneumoniae* (n=40) Resistant (%)	Overall (n=100) Resistant (%)
Monobactam	Aztreonam (ATM)	59 (98.3%)	39 (97.5%)	98 (98%)
Aminoglycoside	Amikacin (AK)	15 (25%)	13 (32.5%)	28 (28%)
Quinolones	Ciprofloxacin (CIP)	59 (98.3%)	38 (95%)	97 (97%)
	Levofloxacin (LEV)	58 (96.7%)	38 (95%)	96 (96%)
Cephalosporins	Cefepime (FEP)	60 (100%)	40 (100%)	100 (100%)
	Cefotaxime (CTX)	60 (100%)	40 (100%)	100 (100%)
Carbapenem	Imipenem (IPM)	24 (40%)	18 (45%)	42 (42%)
β-lactam/β-lactamase inhibitor	Piperacillin/Tazobactam (TZP)	58 (96.7%)	37 (92.5%)	95 (95%)
Polymyxin	Colistin (CT)	60 (100%)	40	

DISCUSSION

The clinical management of Gram-negative bacterial infections faces a severe challenge due to the rapid spread of the mcr-1 gene, a plasmid encoded determinant that confers resistance to the vital antibiotic colistin, thereby depleting our remaining therapeutic options. This study provides critical in vitro evidence that Manuka honey possesses potent, dose-dependent, and bactericidal activity against clinical UTI isolates of *E. coli* and *K. pneumoniae* that are not only multidrug-resistant but also harbor the mcr-1 gene (21).

The antibiotic susceptibility profile of our isolates underscores the gravity of the situation. The 100% resistance to colistin, conferred by the confirmed presence of the mcr-1 gene, aligns with the gene's known mechanism of modifying lipid A, preventing colistin binding (22). Furthermore, the extensive drug resistance observed, including universal resistance to cephalosporins (cefepime, cefotaxime) and high resistance to other classes like quinolones and monobactams, paints a picture of pan-drug resistant (PDR) pathogens. The moderate resistance to imipenem (42%) is particularly alarming, as it signals the erosion of carbapenems, another last-line defense. In this context, the potent antibacterial activity of Manuka honey against such formidable pathogens is highly significant.

Table II. Antibacterial activity of Manuka honey against MCR-1 positive clinical isolates

Bacterial isolates	Agar well diffusion: Zone of inhibition (mm, Mean ± SD)		MIC (% v/v)	MBC (% v/v)
	20% v/v Honey	40% v/v Honey		
E. coli (n=60)	12.1 ± 0.5	18.2 ± 0.8	18	22
K. pneumoniae (n=40)	11.8 ± 0.7	17.5 ± 0.6	22	25
p-value	> 0.05	> 0.05	> 0.05	>0.05

Manuka honey is recognized for its antibacterial properties against a wide range of multidrug-resistant (MDR) pathogens. However, there is limited data on its efficacy against *MCR-1* producing bacteria. In this study, Manuka honey exhibited significant antibacterial activity against all tested *MCR-1* bacteria. Our results demonstrate that Manuka honey (UMF 15+) is effective at concentrations that are clinically attainable, particularly for topical or localized applications like urinary bladder instillation. The mean zones of inhibition of 18.2 mm and 17.5 mm for *E. coli* and *K. pneumoniae*, respectively, at a 40% concentration, confirm a strong antibacterial effect. More importantly, the MIC (18% for *E. coli*, 22% for *K. pneumoniae*) and MBC (22% for *E. coli*, 25% for *K. pneumoniae*) values establish that Manuka honey is not merely inhibitory but decisively bactericidal against these colistin-resistant strains. The close proximity of the MIC and MBC values is a hallmark of a bactericidal agent, suggesting that Manuka honey disrupts essential bacterial functions to the point of cell death. The lack of a statistically significant difference in susceptibility between the two species ($p > 0.05$) indicates that the antibacterial mechanism of Manuka honey is effective across species boundaries and is not circumvented by the specific resistance mechanisms, including MCR-1, that differentiate these pathogens.

Our findings are consistent with, yet critically extend, the existing body of literature. While previous studies have reported Manuka honey's activity against other MDR Gram-negative bacteria, including NDM-1-producing *K. pneumoniae* and various members of the Enterobacterales order, data on MCR-1-harboring isolates from UTIs was scarce (23-25).

When compared to other natural antimicrobials like propolis or garlic extract, Manuka honey's advantage lies in its standardized potency, driven by its defined methylglyoxal (MGO) content, and its multi-faceted mechanism of action. This complex, non-specific targeting presents a significantly higher barrier to resistance than the single-target mechanisms of many other natural compounds (26).

Unlike synthetic antibiotics, microorganisms are unable to develop resistance to honey, making it a unique and effective antimicrobial agent. The chemical composition of honey plays a significant role in its antimicrobial activity. In general, honey exhibits antioxidant properties and contains bioactive compounds such as polyphenols, flavonoids, and vitamin C, which contribute to its ability to combat pathogens (27, 28).

The efficacy of different honeys against resistant pathogens is supported by multiple studies across various regions. A clinical trial in Pakistan established that Beri honey is effective in promoting wound healing and significantly lowering bacterial load (29). In laboratory settings, one investigation found that Manuka honey created a zone of inhibition measuring 7.4 mm against NDM-1-positive Gram-negative bacteria from blood samples (30). Our own results, which identified MIC values of 18-22% for Manuka honey against mcr-1-harboring *E. coli* and *K. pneumoniae*, are corroborated by recent international research. These findings show close agreement with a US study that documented MICs of 21-27% for Manuka honey against Enterobacterales (31). Further reinforcing this, a separate Pakistani study reported that a 30% (v/v) concentration of Manuka honey was bactericidal against NDM-1-producing *K. pneumoniae* (32). An Indian study on *Apis indica* honey also reported potent activity, demonstrating bactericidal effects against *S. typhi* at a much lower concentration of 3% (v/v) (33). Similarly, studies from Ireland and the UK have documented its strong effect against MDR biofilms, which are often implicated in chronic and relapsing UTIs (34-37). Our work specifically bridges the gap by confirming that this efficacy robustly translates to the dire clinical scenario of colistin-resistant, MCR-1 positive UTI pathogens.

CONCLUSION

In conclusion, this study unequivocally demonstrates that Manuka honey is a potent bactericidal agent against colistin-resistant, MCR-1 positive *E. coli* and *K. pneumoniae*. Its ability to overcome a key last-line resistance mechanism via a multi-faceted mode of action makes it a compelling candidate for development as an alternative or adjunctive therapeutic strategy. These promising in vitro findings warrant further investigation into practical clinical applications, such as the development of topical formulations for catheter-associated UTIs or bladder instillation therapies for recalcitrant infections, where localized delivery is feasible. In the face of the looming post-antibiotic era, natural products like Manuka honey offer a beacon of hope, and their rigorous scientific evaluation is not just warranted but essential.

Study limitations:

Despite these promising results, our study has limitations. The in vitro nature of this investigation means the findings require validation in clinical settings. Furthermore, the single-center design and relatively small sample size may limit the generalizability of our results. Future studies should include multi-center collaborations with larger, diverse sample collections, evaluate the anti-biofilm efficacy of Manuka honey against these specific MCR-1 isolates, investigate its synergistic potential when combined with conventional antibiotics, and assess its safety and efficacy in in vivo models of UTI.

Conflict of interest:

Authors have no conflict of interest.

Authors' contribution:

AA, AS & HN Conceptualized, supervision, and writing; AJ, AA, FR, ZK, AW & AG Data collection and writing; AA, SHA, AJ, AW & RA Writing, review; AA, AS & AJ Critical analysis; RA Data collection and writing; AA, HN & AJ Statistical analysis.

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