Journal of Biomedical Sciences | ISSN: 2960-9356
Research Article Peer-review, Open Access

Detection of the ureC gene and its Association with Biofilm Formation in Proteus Mirabilis Isolated from Patients with Type 2 Diabetes Mellitus

Kadhim Kamil Kadhim [1]

Saif Ismael Hussein [2]

Wisam Jasim Mohammed [3]

Abstract: Introduction: Urinary tract infections caused by Proteus mirabilis are increasingly associated with antimicrobial resistance, biofilm formation, and ureC virulence gene expression, especially in diabetic patients. **Aim**: To detect the ureC gene and evaluate its association with biofilm formation and antimicrobial resistance in Proteus mirabilis isolated from UTI patients with type 2 diabetes mellitus. **Materials & Methods**: This study was conducted at Azadi Teaching Hospital, Kirkuk, Iraq. A total of 187 urine samples were collected from type 2 diabetic patients diagnosed with urinary tract infections between November 2025 and February 2026. Standard microbiological, biochemical, antimicrobial susceptibility testing, biofilm detection, and PCR techniques were performed for identification and molecular analysis of Proteus mirabilis isolates. **Results**: A total of 187 urine samples were collected from type 2 diabetic patients with urinary tract infections, of which 17 (9.1%) were positive for Proteus mirabilis. Antimicrobial susceptibility testing revealed high resistance rates against Piperacillin (94.1%), Clindamycin (88.2%), Trimethoprim (82.4%), and Trimethoprim/Sulfamethoxazole (76.5%), while high susceptibility was observed for Tobramycin (94.1%) and Imipenem (88.2%). Among the isolates, 64.7% were classified as multidrug-resistant (MDR), 23.5% as extensively drug-resistant (XDR), and 11.8% as pan-drug resistant (PDR). Biofilm formation analysis showed that 94.1% of isolates were biofilm producers, including 23.5% strong, 41.2% moderate, and 29.4% weak producers, while only 5.9% were non-biofilm formers. Molecular detection demonstrated that the ureC gene was present in 82.4% (14/17) of P. mirabilis isolates. **Conclusion**s: Proteus mirabilis isolates from diabetic UTI patients showed high virulence, strong biofilm formation, and significant multidrug resistance associated with ureC gene presence.

Keywords: Proteus mirabilis; UTI; biofilm; ureC gene; multidrug resistance.

[1] Babylon Directorate of Education, Ministry of Education, Iraq.

[2] Kirkuk Directorate of Education, Ministry of Education, Iraq.

[3] Department of Forensic Evidence Techniques, Al-Hawija Polytechnic College, Northern Technical University

Kadhim.k.kadhim@gmail.com

World of Medicine: Journal of Biomedical Sciences Vol .3 No.11 (2026)
https://wom.semanticjournals.org/index.php/biomed

Introduction

Urinary tract infections (UTIs) are the most prevalent and consequential bacterial infections globally and we are faced with a significant public health challenge due to the very high prevalence, significant recurrence and the increasing emergence of antimicrobial resistance. While Escherichia coli continues to be the most prevalent pathogen causing uncomplicated UTIs, Proteus mirabilis is considered a significant opportunistic pathogen for complicated urinary tract infections, particularly in hospitalized and catheterized patients[1][2]. Both a wide array of virulence factors, such as swarming motility, fimbrial adhesion, hemolysin production, proteolytic enzymes, urease and biofilm formation, all contributing to colonization, persistence and damage to host tissue, has been implicated in the pathogenic success of P. mirabilis. Owing to P. mirabilis' environmental adaptability and in situ adherence in the urinary tract, it can be linked to complicated urinary tract infections. In host defense mechanisms, this organism is significant, especially among patients with extreme illnesses these as sort 2 diabetic issues mellitus (T2DM). They have lower function of neutrophil, chemotaxis, impaired phagocytic activity and reduced intracellular killing in Diabetic patients[3]. Moreover, hyperglycemia and glucosuria provide a nutrient-rich environment for bacteria, and diabetic neuropathy may cause bladder dysfunction, urinary retention, and incomplete emptying, all of which increase risk of infection and re-infection[4]. Summarization-> One of the major virulence factors secreted by P. mirabilis is urease — a nickel dependent enzyme which hydrolyzes urea to ammonia and carbon dioxide, leading to the alkalinization of urine. It promotes precipitation of magnesium ammonium phosphate and calcium phosphate crystals and contributes to both stone formation and crystalline biofilm development. These crystalline forms protect bacteria from host immune responses and promote retention in the urinary tract. UreC, the structural gene, encodes the best-proven subunit of urease, and it is essential for urease activity and the bacterial virulence[5]. One of the most important survival strategies of P. mirabilis is biofilm formation, which plays an important role in chronic urinary tract infections. Biofilms are defined as structured microbial communities in an extracellular polymeric matrix of polysaccharides, proteins, and extracellular DNA. This matrix enhances the adhesion of bacteria to urinary tract tissues and medical devices, such as catheters, and protects bacteria from antibiotics and host immune defenses. As a result, infections that are biofilm-associated have a strong resistance to anti-infective agents and lead to chronic and recurrent infections[6][7][8]. The correlation between urease activity and formation of biofilm is known to be a central aspect in P. mirabilis pathogenesis. Alkalinization by urease, leading to crystal generation, followed by incorporation of crystals into the biofilm matrix, defines crystalline biofilms that block urinary catheters, causing chronic infection. Crystalline biofilms are a signature of complex P. mirabilis infections and highly linked to unsuccessful treatment and urinary blockage[9]. Another aspect associated with biofilm formation is antimicrobial resistance. The basis for this tolerance can be attributed to incomplete penetration of antibiotics, a decrease in the metabolic activity of biofilm-residing bacteria and the confinement of persister cells. This allows for persistent infections which are difficult to treat, even with appropriate antibiotic therapy[10]. Numerous studies reveal that biofilm producing P. mirabilis isolates exhibit remarkably elevated rate of resistance to multiple antibiotics when compared to the non-biofilm producers[11]. Moreover, virulence factors reported to correlate with multidrug resistance in clinical isolates are associated with the relationship between pathogenicity and antimicrobial resistance[12][13]. The significance of urease genes on the pathogenicity of P. mirabilis has been confirmed by molecular studies. Ub 40 years ago, the organization and expression of ureD and ureC were demonstrated early by genetic analysis, as was a predominant role of the purified enzyme within the urea cycle. Further clinical relevance for urease in urological infections and stone formation and persistence was confirmed in subsequent studies. Recent studies have highlighted the multifactorial component of P. mirabilis virulence, as well as the importance of biofilm-associated gene expression in clinical isolates[14][15]. Although there is extensive research regarding P.mirabilis, little has been published regarding the association of the ureC gene and the formation of biofilm with isolates from patients with

type 2 diabetes mellitus who suffered urinary tract infections, especially in Iraq. Thus this study aimed to identify ureC in Proteus mirabilis isolates from UTI in subjects with type 2 DM in relation to biofilm formation.

Methodology

Study Design and Sample Collection

A cross-sectional study was performed at Azadi Teaching Hospital, Kirkuk, Iraq between November 2025 and February 2026. They comprised a total of 187 midstream urine specimens collected from type 2 diabetes mellitus patients with a clinical diagnosis of urinary tract infections (UTIs). Patient recruitment was done on the basis of clinical evaluation by specialist physicians including medical history, presenting symptoms and routine laboratory test results. All the urine samples were taken in a sterile leak-proof container with aseptic notes and transported immediately to the microbiology laboratory for processing.

Inclusion and Exclusion Criteria

Patients with type 2 diabetes mellitus who had clinical symptoms suggestive of UTIs were enrolled. We excluded patients with a history of systemic antibiotic therapy within two weeks before specimen collection (n = 280), those with contaminated urine sampling (n = 183), and patients with insufficient cases (n = 12).

Isolation and Cultivation of Bacterial Isolates

Urine samples had been processed utilizing the semi-quantitative culture strategy immediately following collection. Blood agar and MacConkey agar plates were made for each specimen, using a calibrated sterile loop (0.001 mL). Inoculated plates were then aerobically incubated at 37 °C for 18–24 hours. After incubation with bacterial colony size, colony morphology as well as lactose fermentation characteristics and hemolytic activity were assessed.

Identification of *Proteus mirabilis*

The bacterial isolates were screened by routine microbiological and biochemical identification methods. Colonies showing typical swarming motility on Blood agar and non-lactose fermenting colonies on MacConkey agar were chosen for further study. Gram staining demonstrated Gram-negative bacilli. Biochemical characterization consisted of urease, indole, methyl red (MR), and Voges–Proskauer (VP) tests. Identificaste proteus mirabilis presumible isolates that are positive urease and MR reactions and negative indole and VP reactions. Confirmation at species level was independently performed using VITEK® automated identification system.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility profiles of all confirmed P. mirabilis isolates were evaluated by the Kirby–Bauer disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines on Mueller–Hinton agar. Before inoculation, the fresh bacterial suspensions were adjusted to the turbidity equivalent to a 0.5 McFarland standard. Antibiotic discs were placed onto the surface of agar that had been inoculated with the organism and plates were left to incubate aerobically at 37°C for 18-24 hours. Inhibition zones were measured and classified accordingly as susceptible, intermediate or resistant based on CLSI breakpoints.

Detection of Biofilm Formation

The ability to form biofilm was assessed by microtiter plate (MTP) assay. Overnight cultures of bacteria were diluted in the appropriate growth medium and inoculated into sterile 96-well polystyrene microplates. After incubation at 37 °C for 24 h, planktonic cells were removed, and wells were washed three times with phosphate-buffered saline (PBS). Adherent biofilms were fixed with 2.5% glutaraldehyde and subsequently stained with 0.1% crystal violet, after which excess stain was thoroughly washed off. Following this, bound dye was solubilized with ethanol, and optical density (OD) of the biofilm biomass was measured using a microplate reader.

Molecular Detection of the *ureC* Gene

Preparation of Bacterial Pellets

By incubating fresh bacterial colonies in nutrient broth overnight at 37 °C with constant stirring. The cultures were then centrifuged at 4000 rpm for 10 mins, after which the supernatant was removed. The harvested bacterial pellets were stored at −20°C prior to genomic DNA extraction.

Genomic DNA Extraction

The genomic DNA was extracted using the Geneaid Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan) according to the instruction of the manufacturer. In short, bacterial cells were lysed and DNA was purified by a silica-based spin-column. Purified DNA was eluted in the provided elution buffer and stored at −20° until PCR analysis.

PCR Amplification of the *ureC* Gene

Conventional polymerase chain reaction (PCR) was then used to amplify the ureC gene. Consensus: Each 25 µL reaction mixture contained 12.5 µL PCR Master Mix, 1 µL forward Primer, 1 µL reverse Primer, 2 µL template DNA and 8.5 µL nuclease-free water. The ureC gene was amplified using a programmable thermal cycler with optimized cycling conditions.

Agarose Gel Electrophoresis

Electrophoresis of the PCR products were done on 1.5% agarose gel in 1× TBE buffer. Each 5 µL of amplicon mixed with loading dye was loaded to the gel, together with a 100 bp DNA ladder. Electrophoresis was performed for around 30 min at 230 V. After ethidium bromide staining and UV illumination, the PCR fragments were visualized and the expected amplicon sizes were determined by comparison with the molecular weight marker.

Table 1. Primers used for PCR amplification of the *ureC* gene

Gene	Primer sequence (5′–3′)	Product size (bp)	Ref.
ureC	F: GTTATTCGTGATGGTATGGG	316	(16)
	R: GTAAAGGTGGTTACGCCAGA		

Statistical Analysis

Statistical Package for the Social Sciences SPSS software version 19 and data were analyzed using. Descriptive statistics were expressed as frequencies and percentages, or mean ± standard error (SE) whenever applicable. Statistical comparisons between groups were performed using the independent-samples t-test or one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for post hoc comparisons. Statistical significance was defined as a P value of <0.05.

Results & Discussion

Isolation of *Proteus mirabilis*

A total of 187 urine samples were collected from diabetic patients suffering from urinary tract infections. Among these samples, 17 isolates were identified as *Proteus mirabilis*, whereas 160 samples were negative. The overall isolation rate of *P. mirabilis* was 9.1%, Table 2 & figure 1.

Table 2. Isolation rate of *Proteus mirabilis* from urine samples

Result	Number of Samples	Percentage (%)
Positive for *P. mirabilis*	17	9.1
Negative for *P. mirabilis*	160	90.9
Total	187	100%



Figure 1. *P. mirabilis* colonies on MacConkey agar

In the current study, the isolation rate of Proteus mirabilis was 9.1% in diabetic patients with urinary tract infections. This results similar to the study conducted by[16] Al-Fahham Kareem found 8.7% of isolation rate among Iraqi UTI patients. They ascribed to the ubiquity of P. mirabilis, capable of colonizing the urinary tract via its urease production, swarming motility, and fimbrial adhesion. In their investigation, Kareem and Alkhafaji[17] also showed that P. mirabilis constituted around 10% of urinary isolates, indicating that immune dysfunction and hyperglycemia induced by diabetes promote bacterial colonization and infection.

Antibiotic susceptibility

The antibiotic susceptibility pattern for Proteus mirabilis isolates (n = 17) was varied (Table 3). Ampicillin (AM 25) was completely effective, all isolates were sensitive 17 (100.0%) and no intermediate or resistant strains were detected. However, Piperacillin (PRL 100) showed the highest resistance rates (16 (94.1%) resistant and 1 (5.9%) intermediate). Clindamycin (DA 10) also had low activity against these isolates with 15 (88.2%) resistant strains, but 1 (5.9%) sensitive and 1 (5.9%) intermediate. Trimethoprim (TMP 10) also showed the high rates of resistance with a total of 14 (82.4%) resistant isolates, against 2 (11.8%) sensitive and 1 (5.9%) intermediate isolates. Trimethoprim/Sulfamethoxazole (SXT 25), [13 (76.5%) resistant, 3 (17.6%) sensitive, 1 (5.9%) intermediate] About ceftriaxone (CRO 30), moderate resistance was also shown, and this test had 10 (58.8%) resistant isolates, 5 (29.4%) sensitive and 2 (11.8%) intermediates. Likewise gentamicin (CN 10) 11(64.7%) isolates were resistant, 4 (23.5%) sensitive, and 2 (11.8%) intermediates; thus gentamicin was also less effective. Chloramphenicol (C 30) showed moderate activity with 14 (82.4%) sensitive and 3 (17.6%) resistant isolates, respectively. No resistant isolates were detected with imipenem (IPM 10), which exhibited the highest efficacy among all tested antibiotics (15 [88.2%] sensitive isolates and 2 [11.8%] intermediates). In the same way, Tobramycin (TOB 10) presented notable antibacterial activity, 16 (94.1%) sensitive isolates and 1 (5.9%) intermediate, with absence of resistance, (Table 3, Figure 2).

Table 3. Antibiotic susceptibility pattern of *Proteus mirabilis* isolates (n = 17)

Antibiotic	Sensitive	Intermediate	Resistant
Ampicillin (AM 25)	17 (100.0%)	0 (0.0%)	0 (0.0%)
Piperacillin (PRL 100)	0 (0.0%)	1 (5.9%)	16 (94.1%)
Clindamycin (DA 10)	1 (5.9%)	1 (5.9%)	15 (88.2%)
Trimethoprim (TMP 10)	2 (11.8%)	1 (5.9%)	14 (82.4%)
Trimethoprim/ Sulfamethoxazole (SXT 25)	3 (17.6%)	1 (5.9%)	13 (76.5%)
Ceftriaxone (CRO 30)	5 (29.4%)	2 (11.8%)	10 (58.8%)
Gentamicin (CN 10)	4 (23.5%)	2 (11.8%)	11 (64.7%)
Chloramphenicol (C 30)	14 (82.4%)	0 (0.0%)	3 (17.6%)

Imipenem (IPM 10)	15 (88.2%)	2 (11.8%)	0 (0.0%)
Tobramycin (TOB 10)	16 (94.1%)	1 (5.9%)	0 (0.0%)

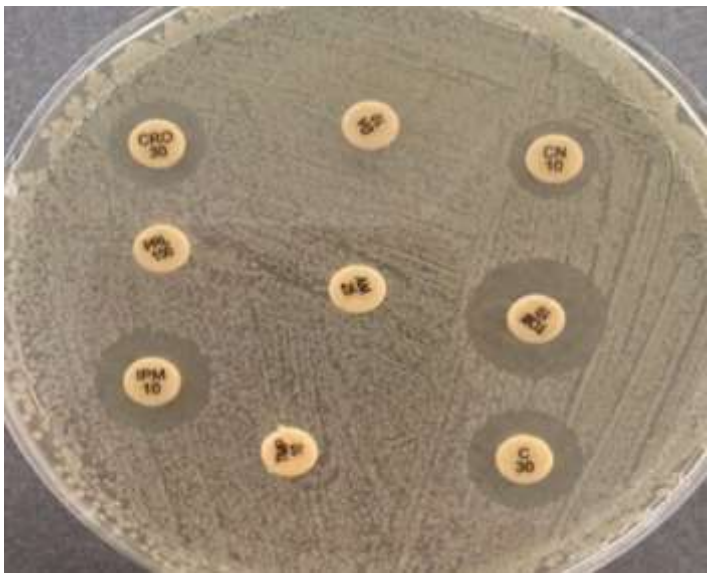


Figure 2. Antibiotic susceptibility of *Proteus mirabilis* isolates on Mueller-Hinton Agar.

Out of 17 isolates, 11 (64.7%) were identified as MDR, indicating resistance to at least one agent in three or more antimicrobial categories. Additionally, 4 isolates (23.5%) were categorized as extensively drug-resistant (XDR), showing susceptibility to only limited antimicrobial options. Furthermore, 2 isolates (11.8%) were classified as pan-drug resistant (PDR/EDR), demonstrating resistance to nearly all tested antibiotics, Table 4.

Table 4. Classification of *Proteus mirabilis* isolates based on resistance pattern

Category	Number of isolates	Percentage (%)
MDR (Multidrug Resistant)	11	64.7%
XDR (Extensively Drug Resistant)	4	23.5%
PDR / EDR (Pan Drug Resistant)	2	11.8%
Total	17	100%

The observed high resistance to β-lactam antibiotics was also reported by Kadhim[18], where P. mirabilis isolates exhibited resistance levels >80–100% for penicillins and cephalosporins. They imputed this to the production of β-lactamase and the widespread misuse of antibiotics in clinical practice that favors the selection of resistant strains. Likewise, resistance to Trimethoprim/Sulfamethoxazole (76.5%) is in agreement with reports by Khoshaba[19], with rates of resistance of >70% for clinical P. mirabilis isolates. The reason for this is that this drug is often used empirically in urinary tract infections, resulting in significant selective pressure and the appearance of strains containing resistant genes. The moderate resistance agreement to Gentamicin(64.7%) is similar with Abed-Gumar[20], Aminoglycoside resistance in P. mirabilis isolates ranged from 60–75% in the current study compared to a previous study. This resistance is primarily linked to aminoglycoside-modifying enzymes and reduced permeability of the bacterial membrane keeping intracellular antibiotic accumulation low. In comparison, the lower rates for Imipenem showed a high susceptibility to this phenotype (88.2%), consistent with Hammoudi[21], who demonstrated carbapenem susceptibility in the Enterobacterales isolates above 80%. Due to irrepressibility by majority types of β-lactamases, carbapenems continue to be one of the most potent antimicrobials, but the emergence of resistance has become an increasing problem. Like-wise, Tobramycin had the prominent activity (94.1%); in agreement with Jacobsen[22], who showed that aminoglycosides have preserved efficacy against P. mirabilis, attributed to their bactericidal action and the low prevalence of resistance among cultures from community patients. The results emerged from this study were similar with the study

of Wasfi with the isolation of MDR, XDR and PDR isolates. Prow bow biofilm development and horizontal data transfer contribute to antimicrobial resistance in the treatment failure[23]. Our finding supports the role of the immune dysfunction in diabetic patients to rescue the bacteria, thus underscoring the clinical challenge of persistent urinary tract infections.

Biofilm Formation

Out of the total isolates, 4 (23.5%) were strong biofilm producers, 7 (41.2%) showed moderate biofilm formation, and 5 (29.4%) were weak biofilm producers. In addition, 1 isolate (5.9%) was classified as a non-biofilm producer Table 5.

Table 5. Biofilm formation ability of *Proteus mirabilis* isolates

Biofilm Formation Type	Number of Isolates	Percentage (%)
Strong	4	23.5%
Moderate	7	41.2%
Weak	5	29.4%
Non-biofilm producers	1	5.9%
Total	17	100%

Our present findings agree with Flores-Mireles[24] who showed that about 80-90% of uropathogenic bacteria, including P. mirabilis, can form biofilms in urinary tract environments. They highlighted that bacterial biofilm formation is responsible for clinical persistence and for resistance against a range of antimicrobial agents. This observation, compared with our results (94.1% biofilm-positive isolates) suggests better adaptation properties of the local strains as reflected on biofilm-forming potential. Similarly, approximately 85% of clinical P. mirabilis isolates have been previously reported to produce biofilms according to Wasfi, with 35% of this classified as strong producers and 30% as moderate biofilm producers. By contrast, our results showed 23.5% strong and 41.2% moderate biofilm producers which would suggest that our isolates profile a higher proportion of moderate biofilm formers than strong biofilm formers, reminiscent of the effect of different selection pressures experienced by our isolates as a result of host conditions (diabetic patients), antibiotic exposure and environmental pressure. Jacobsen, Crystalline biofilm formation is strongly associated with catheter-associated P. mirabilis infections, with nearly all catheterized isolates being biofilm-related colonization. No gliding-associated catheter-associated infections were studied in our study, but the very high overall biofilm rate (94.1%) is in line with their studies and corroborates that P. mirabilis seems to have an intrinsic capacity to form biofilms in the uropathogenic environment. Furthermore, Yang Sok[25] found that 70–80% of P. mirabilis clinical isolates were strongly positive for biofilm and approximately 25% of them could be classified as strong biofilm producers. These values are remarkably consistent with our results (strong producers, 23.5%, indicating a high degree of consistency of overall agreement of our results with global data).

Detection of ureC

The molecular detection of virulence determinants demonstrated that the *ureC* gene was detected in 14 out of 17 *Proteus mirabilis* isolates, yielding a positivity rate of 82.4% (Figure 3).

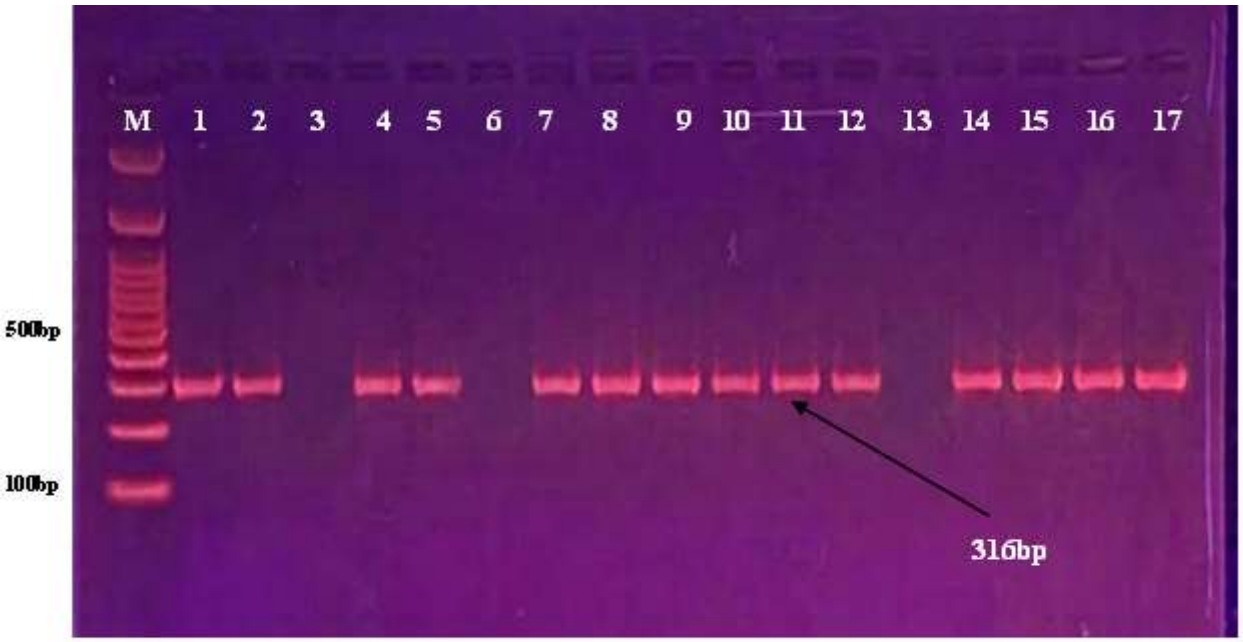


Figure (3): PCR amplification of 316bp *ureC* gene by 1.4% agarose gel electrophoresis.
Ladder: M, Lane (1-17): PCR product of *P. mirabilis* isolates from urine samples

The achieved results coincide with Ahmad et al. Catheter-associated urinary tract infections also carried a high detection rate of ureC, with positivity exceeding 80% of clinical isolates, in the study of[26]. They then found that the ureC gene was important for increased urease activity of the enzyme generalisation level, which increased urine alkaline guidelines to support crystal formation to enhance bacterial survival and chronic infection. In a similar study, ureA associated genes in clinical isolates of P. mirabilis were detected at high frequencies by Kareem and Alkhafaji, concluding that urease gene clusters are highly conserved in uropathogenic strains. Their study also showed that urease gene expression plays an important role in the establishment of colonization of the urinary tract by bacteria and in biofilm associated infections. Moreover, the ureR regulatory gene was detected in some of P. mirabilis isolates implicated in urinary tract infection in Najaf, Iraq by Al-Fahham and Kareem. They showed that the regulatory genes of urease are correlated with rapid urease activity and enhanced virulence. The proportions of the detection in their study confirmed the notion that urease gene systems constitute an important virulence determinant in these infections of P. mirabilis.

Conclusions

The investigation shows that Proteus mirabilis is a clinically important uropathogen in diabetic patients with high antimicrobial resistance rates, and demonstrated significant biofilm formation and ureC virulence gene prevalence. The simultaneous presence of the MDR, XDR and PDR phenotypes in addition to biofilm production indicates that this organism has a high adaptability and survival ability in the urinary tract ecosystem. The urgency of continuous antimicrobial surveillance, rational antibiotic use, and strategies targeting virulence factors to improve infection control and treatment outcomes is highlighted.

References

[1] C. E. Armbruster and H. L. T. Mobley, “Merging mythology and morphology: The multifaceted lifestyle of *Proteus mirabilis*,” *Nature Reviews Microbiology*, vol. 10, no. 11, pp. 743–754, 2012.

[2] S. M. Jacobsen, D. J. Stickler, H. L. T. Mobley, and M. E. Shirtliff, “Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*,” *Clinical Microbiology Reviews*, vol. 21, no. 1, pp. 26–59, 2008.

[3] J. N. Schaffer and M. M. Pearson, "*Proteus mirabilis* and urinary tract infections," *Microbiology Spectrum*, vol. 3, no. 5, Art. no. UTI-0017-2013, 2015.

[4] B. D. Jones and H. L. T. Mobley, "*Proteus mirabilis* urease: Genetic organization, regulation, and expression of structural genes," *Journal of Bacteriology*, vol. 170, no. 8, pp. 3342–3349, 1988.

[5] D. J. Stickler, "Clinical complications of urinary catheters caused by crystalline biofilms: Something needs to be done," *Journal of Internal Medicine*, vol. 276, no. 2, pp. 120–129, 2014.

[6] J. W. Costerton, P. S. Stewart, and E. P. Greenberg, "Bacterial biofilms: A common cause of persistent infections," *Science*, vol. 284, no. 5418, pp. 1318–1322, 1999.

[7] L. Hall-Stoodley, J. W. Costerton, and P. Stoodley, "Bacterial biofilms: From the natural environment to infectious diseases," *Nature Reviews Microbiology*, vol. 2, no. 2, pp. 95–108, 2004.

[8] R. Wasfi, S. M. Hamed, M. A. Amer, and L. I. Fahmy, "*Proteus mirabilis* biofilm: Development and therapeutic strategies," *Frontiers in Cellular and Infection Microbiology*, vol. 10, Art. no. 414, 2020.

[9] P. S. Stewart and J. W. Costerton, "Antibiotic resistance of bacteria in biofilms," *The Lancet*, vol. 358, no. 9276, pp. 135–138, 2001.

[10] R. A. Flamerz, S. S. Obid, and W. M. Jasim, "Study the effect of biofilm production on antibiotic resistance in *Proteus mirabilis* isolated from clinical samples in Kirkuk City," *NTU Journal of Pure Sciences*, vol. 2, no. 1, pp. 61–70, 2022.

[11] M. A. E. El-Sayed Ahmed *et al*., "A correlation study between virulence factors and multidrug resistance among clinical isolates of *Proteus mirabilis*," *Brazilian Journal of Microbiology*, vol. 54, pp. 1765–1777, 2023.

[12] K. S. Liao *et al*., "Quorum sensing systems in *Proteus mirabilis* and their role in biofilm formation and virulence," *Microbiological Research*, vol. 258, Art. no. 126993, 2022.

[13] M. C. Nissanka, A. Dilhari, J. A. Munasinghe, K. Bandara, I. Munaweera, *et al*., "Decoding *Proteus mirabilis* biofilms: Expression of selected virulence genes and association with antibiotic resistance," *BMC Microbiology*, vol. 25, no. 1, Art. no. 481, 2025.

[14] Z. Sun *et al*., "The role of *luxS* in quorum sensing and biofilm formation in bacteria," *Future Microbiology*, vol. 9, no. 3, pp. 281–294, 2014.

[15] W. D. Fahem, S. N. Abdullah, and H. A. R. Abbood, "A study on quorum sensing gene *luxS* and mixed biofilm dynamics of *Proteus mirabilis* and *Candida albicans* in urinary tract infections," *Basrah Journal of Science*, vol. 44, no. 1, 2026.

[16] H. R. A. Al-Fahham and R. K. Kareem, "Molecular study of urease *ureR* gene of *Proteus mirabilis* isolated from urinary tract infections, Najaf, Iraq," *Archives of Razi Institute*, vol. 77, no. 3, pp. 1257–1260, 2022, doi: 10.22092/ARI.2022.357465.2042.

[17] R. Kareem Shameel and H. M. Alkhafaji, "Molecular detection of the *hpmA* and *ureA* genes in clinical *Proteus mirabilis* isolates," *Ibn Al-Haitham Journal for Pure and Applied Sciences*, vol. 38, no. 1, pp. 50–59, Apr. 2025, doi: 10.30526/38.1.3545.

[18] A. S. Kadhim, "Antimicrobial resistance patterns and ESBL production in *Proteus mirabilis* isolated from clinical samples," *Al-Mustansiriyah Journal of Science*, vol. 28, no. 1, pp. 47–54, 2017, doi: 10.23851/mjs.v28i1.311.

[19] R. Khoshaba, "The prevalence of extended spectrum beta-lactamase genes SHV, TEM and CTX-M in clinical isolates of *Proteus mirabilis*," *Iraqi Journal of Science*, vol. 66, no. 11, 2025, doi: 10.24996/ijs.2025.66.11.19.

[20] E. Abed-Gumar, A. Salim Hamzah, and W. Fadhil Hamad, "Study of some resistance genes in clinical *Proteus mirabilis*," *Archives of Razi Institute*, vol. 77, no. 6, pp. 2235–2242, Dec. 2022, doi: 10.22092/ARI.2022.358489.2230.

[21] A. A. Hammoudi, "Antibiotics susceptibility pattern of some Enterobacteriaceae isolates from different clinical infectious sources," *International Journal of Research in Pharmaceutical Sciences*, vol. 10, no. 1, pp. 737–744, Feb. 2019, doi: 10.26452/ijrps.v10i1.1914.

[22] S. M. Jacobsen, D. J. Stickler, H. L. T. Mobley, and M. E. Shirtliff, "Complicated catheter-associated urinary tract infections due to *Proteus mirabilis*," *Clinical Microbiology Reviews*, vol. 21, no. 1, pp. 26–59, 2008.

[23] R. Wasfi, S. M. Hamed, M. A. Amer, and L. I. Fahmy, "*Proteus mirabilis* biofilm: Development and therapeutic strategies," *Frontiers in Cellular and Infection Microbiology*, vol. 10, Art. no. 414, 2020.

[24] A. L. Flores-Mireles, J. N. Walker, M. Caparon, and S. J. Hultgren, "Urinary tract infections: Epidemiology, mechanisms of infection and treatment options," *Nature Reviews Microbiology*, vol. 13, no. 5, pp. 269–284, 2015.

[25] A. Yang, Y. Tian, and X. Li, "Unveiling *Proteus mirabilis* virulence in UTIs," *Frontiers in Cellular and Infection Microbiology*, vol. 14, Art. no. 1465460, 2024.

[26] N. A. Ahmad, N. B. Jafar, and S. H. Mohammed, "Molecular detection of the virulence *ureC* gene of *Proteus mirabilis* in urine samples from catheterized patients," *Kirkuk Journal of Medical Sciences*, vol. 13, no. 2, pp. 46–53, 2025, doi: 10.32894/kjms.2025.160662.1160.