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# **Original** article

# UreC gene detection of Proteus mirabilis isolated and identified from patients with UTI

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#### ABSTRACT

Background: Proteus mirabilis is commonly associated with urinary tract infections (UTIs), and many patients exhibit antibiotic resistance to this bacterium and others. This study aimed to isolate and identify P. mirabilis in patients suffering from UTIs and to investigate the molecular identification of the UreC gene. Methods: A total of 135 clinical samples were collected from patients at Kirkuk General Hospital in Kirkuk City, northern Iraq, between September and December 2024. The samples were collected as midstream urine samples in sterile cups from both sexes across various age groups. Results: The findings indicated that, when urine samples from UTI patients were cultured on blood agar and MacConkey agar, 32 samples (23.7%) tested positive for P. mirabilis. In contrast, 103 samples (76.3%) exhibited negative results for P. mirabilis. The isolates demonstrated significant antibiotic resistance, with 81.3% resistant to Vancomycin, 78.1% to Clindamycin, and 75.0% to Ceftazidime. Additionally, complete resistance (100%) was observed to Ampicillin. However, P. mirabilis showed considerable sensitivity to Amikacin (93.8%) and Imipenem (84.4%), and it exhibited 100% sensitivity to Tobramycin. Genetically, out of the 32 isolates, 19 (59.4%) of the P. mirabilis isolates carried the UreC gene, which acts as a virulence factor. Conclusions: This study found that the *UreC* gene, which codes a large subunit responsible for regulating urease enzyme synthesis in P.mirabilis, is notable for its widespread presence across all species of Proteus.

#### Introduction

Urinary tract Infections (UTIs) where infections or inflammations of any region of the urinary system that are brought on by different bacteria or, occasionally, fungi [1, 2]. It affects over 150 million individuals globally each year and is frequently seen in clinical settings, where it accounts for roughly 10 - 20% of infection cases at primary care centers, while 30 - 40% in clinics. Females are four times more likely than males to develop an infection. UTIs can be broadly classified into two groups: community-associated UTIs (CAUTIs) and

healthcare-associated UTIs (HAUTIs) [3, 4, 5]. *Proteus* bacteria are rod-shaped, facultative anaerobic, and Gram-negative [6].

A highly mobile bacterium, Proteus which mirabilis, belongs to the family: Enterobacteriaceae. Different from Enterobacteriaceae members, P. mirabilis is not a prevalent pathogen that infects normal hosts with UTIs [7]. The two types of UTIs are most commonly associated with increasing infections, in which bacteria gradually take possession of the urinary tract, the urethra, the ureter, the kidneys, and hematogenous infections, which are also known as

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systemic infections. Infections with structural abnormalities, infections with urinary catheters in place, and Proteus strains are more likely to induce the second type of UTI [8]. P. mirabilis is an opportunistic causal agent for respiratory system infections, wounds and burns, eyes, skin, nose, ears, and throat, as well as the infection of gastroenteritis that is caused by consuming contaminated meat or other food [7, 9]. These factors, which included toxins like hemolysin, which functioned in pore formation, biofilm formation, and pathogenicity regulation [11], were important in causing an infection in different urinary tract regions because P.mirabilis possessed several numbers of virulence factors that were essential for causing UTIs [10]. The most researched P. mirabilis fimbriae are the MR/P fimbriae, which may be involved in adherence to the uroepithelium and help an organism cause infection in the urinary tract [12]. The urease enzyme, which acts to encourage the breakdown of urea into ammonia, carbon dioxide, and increases urine pH, is another virulence factor.

Urolithiasis, a disorder that can lead to renal failure, is brought on by this process, which also starts the development of kidney and bladder stones (struvite and apatite) [13, 14]. Therefore, the study aimed to isolate and perform molecular identification of *UreC* as a specific gene of *P. mirabilis* from patients with UTI and might be applied in the future for detecting stones as well as antibiotic resistance by this bacterium.

#### **Materials and Methods**

#### **Ethical Approval**

This research requirement was facilitated based on the scientific cooperation record and prior approval from the Ministry of Health and the Ministry of Higher Education and Scientific Research in Iraq. It was conducted according to the ethical principles established in the Helsinki Protocol and approved by the Scientific Research Ethics Committee of the College of Education for Pure Sciences (Ibn Al-Haitham).

Sample collection from the patients and conducting the necessary examinations in the plane of study were done according to the native ethics. Verbal agreements have been obtained earlier from all participating patients, and patients have been informed of the study objectives.

# **Specimen Collection**

Urine samples were collected from 135 Inpatients and outpatients at the diagnostic clinics of

the Urology Unit at Kirkuk General Hospital, northern Baghdad, Iraq, from September to December 2024. Samples were taken according to the internationally approved scientific approach, and patients were informed of the purpose of the study. Samples were collected in a sterile urine cup from both sexes and different ages; Patients were advised to collect a urine sample after leaving the first drops, to ensure the sample is not contaminated with other bacterial species.

#### Isolation and Identification of P. mirabilis

Initially, Nutrient agar (NA), Blood Agar Base No.2 (BA) from (HiMedia Laboratories Pvt. Ltd-India), and MacConkey agar from (Oxoid Cambridge Company-UK) were prepared according to the instructions manual and then used for isolating and growing bacterial isolates from urine samples by streaking plates of these media and incubation for 18 hours at 37.0°C. All urine samples were streaked on these media, and then the positive results were selected for *P. mirabilis* isolates according to Jarjes 2019[12].

Bacterial isolates that gave a gramnegative stain with fishy smell on NA, Swarming motility type on BA, and non-fermented lactose in MacConkey medium were identified as belonging to the genus Proteus sp. Biochemical tests using Methyl Red and Voges-Proskauer, indicators by (Sigma-Aldrich-USA). The detection of urease, catalase, and oxidase enzyme production and the indole test were done for the isolates based on the principles outlined by Mordi and Momoh [4]. Citrate utilization as sole carbon source was used Citrate Simmons Agar from (HiMedia Lab.Pvt.Ltd,India). Fermentation shape on Kliger's Iron slant Agar (KIA) media from (Oxoid Company-UK)[4,12]. Cambridge Later, VITEK® 2 Compact Automated Systems from (bioMérieux, Inc. Hazelwood, MO, USA), It has been used to facilitate the identification of P. mirabilis due to its role in the rapid and accurate diagnosis of microbiological isolates.

#### **Antibiotic Susceptibility Test (AST)**

All isolates identified as *P. mirabilis* by (Vitek 2 Compact system above) were tested for their Antibiotic susceptibility by distribute the antibiotic discs (Kirby-Bauer technique) after spread the isolates on Muller-Hinton (MH) agar made by (Merck, UK). 11 Antibiotic discs supplied by (Bioanalysis company- Turkey) Included

Ampicillin, Vancomycin, Trimethoprim, Ceftazidime, Gentamicin, Imipenem, Ciprofloxacin, Levofloxacin, Azithromycin, Amikacin and Tobramycin. The inhibition zone for each antibiotic was measured in mm, and then AST was compared to the parameters fixed by the Clinical Laboratory Standards Institute (CLSI, 2020) [13].

#### **Isolating Genomic Bacterial DNA of Isolates**

The genomic DNA isolated from the most systematized isolates using the protocol of the technique (ABIO pure Extraction) by **Tanner** *et.al.*, [14]. The purpose of the Wizard® Genomic DNA Purification Kit is to separate DNA from Gramnegative bacteria.

#### **Amplification of DNA Using the PCR Technique**

The traditional PCR method have been used to detect the UreC gene sequences of P. mirabilis isolates. Using 25µl of the DNA fragments, mixed well with two specific primers, revealed the UreC gene. Lyophilized Forward primers sequence of UreCis (5'-CAAGCCCAAGAAGGTCTCGT-3'), while the sequence of *UreC* gene Reverse is (5'-CAAGATGCTCGTCCACGGTA-3'). The expected length DNA fragment of the UreC gene is around 517 bp [15]. The concentration of primers used was 10 pmol/µl.

The mixture fragments of the *UreC* gene and primers was amplified by PCR Technique after adjusting the system as followed in Mahdi and Al-Deresawi [15], then running electrophoresis using 1.5% the agarose gel stained by the Ethidium Bromide and adjusted the system on 100 volts for 60 minutes with the ladder marker (M: 100-1500 bp).

To distinguish the band size of the PCR product on the agarose gel, electrophoresis is used to either detect the DNA extracted fragments or evaluated the PCR products compared with standard DNA.

#### Results

#### **Distribution and Biochemical Tests of Samples**

The study examined 135 urine samples from patients with urinary tract infections (UTIs), consisting of 93 males and 42 females (see Table 1). The biochemical tests revealed that 32 samples (23.7%) were positive for the growth of *P. mirabilis* when cultured on blood and MacConkey agar, while

103 samples (76.3%) showed no growth of *P. mirabilis*.

Through laboratory work, it became clear that the best differential tests and phenotypic characteristics (Table 1) could be relied upon to diagnose *Proteus mirabilis* isolates such as odor (fishy odor), the inability to ferment lactose in MacConkey medium (Figure 1A), which gives it a pale color, and the characteristic motility in solid media such as Blood Agar (Figure 1B). As for the remaining tests, their positive and negative results were shared with the rest of the bacterial isolates that were isolated during the research. Therefore, the diagnosis of the isolates in this study was confirmed based on the VITEK® 2 Compact system and the selected bacterial isolates that matched 99% or more were considered to be *Proteus mirabilis*.

#### **Antibiotic Susceptibility Test**

The results of the antibiotic resistance test revealed significant variation in the susceptibility of the study isolates to the 11 selected antibiotics (Figure 2). The *P. mirabilis* isolates showed complete resistance (100%) to Ampicillin and high levels of resistance to Imipenem (84.4%), Vancomycin (81.3%), Clindamycin (78.1%), and Ceftazidime (75.0%). Among these antibiotics, Imipenem exhibited the highest resistance rate at 84.4%. In contrast, the studied isolates demonstrated high sensitivity to Amikacin (93.8%) and complete sensitivity to Tobramycin (100.0%), as illustrated in Figure 2.

### Possibility of UreC Gene in Isolates

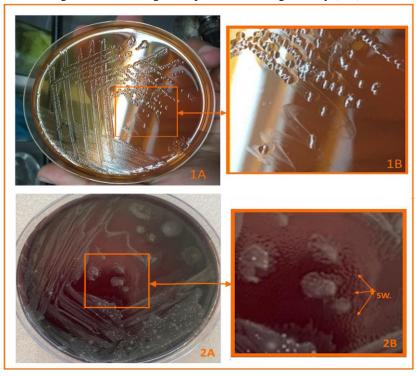
After conducting electrophoresis on the DNA of bacterial isolates identified as *Proteus mirabilis* strains, the results showed that the gel electrophoresis analysis of the *UreC* gene was present in 19 (59.4%) out of 35 genomic DNA samples of the *P. mirabilis* isolates. This gene consists of 517 base pairs, as indicated by the DNA ladder marker used in the study. Figure 3 illustrates the range of *UreC* gene presence in 10 randomly selected DNA samples from the bacterial isolates, confirming the findings of this study.

**Table 1.** Distribution, Morphological characteristics, and Biochemical tests of Bacterial Isolates from UTI clinical samples of the study

Clinical Sources		No. (%) Positive	No. (%) Negative	Total No.(%)
Patients	Male	10(7.4 %)	32(23.7%)	135 (100.0%)
	Female	22(16.2%)	71(52.6%)	
Morphological characteristic	Gram stain	62	40	33 no bacterial growth
	Fishy smell	32	Variable smells	
	Swarming on NA	35 (Variable in intensely)	40	
	Growth on Blood Agar	All bacterial isolates were grown		
Biochemical tests of Isolates	Growth on MacConkey	62	40	Only 35 isolates non- lactose fermented
	Methyl Red	73	29	All P. mirabilis (+)
	Citrate	46	56	All P. mirabilis (+)
	Ureases	69	33	All P. mirabilis (+)
	Catalase	48	54	All P. mirabilis (+)
	Oxidase	38	64	All P. mirabilis (-)
	Indole	22	80	All P. mirabilis (-)
	Voges- Proskauer	24	78	All P. mirabilis (-)
	Growth on KIA*	84*	18	

<sup>\*</sup> KIA= Kliger's Iron Agar, its medium used for differentiation among the bacterial isolates according to their ability to produce H2S, gases, and ferment either glucose or lactose or both during growth by producing different acids.

**Figure 1.** *P. mirabilis* colonies of one isolate, 1A- on MacConkey agar, showing and magnified part in 1B. Growth isolates on Blood Agar in 2A and magnified part of swarming motility (sw.) in 2B.



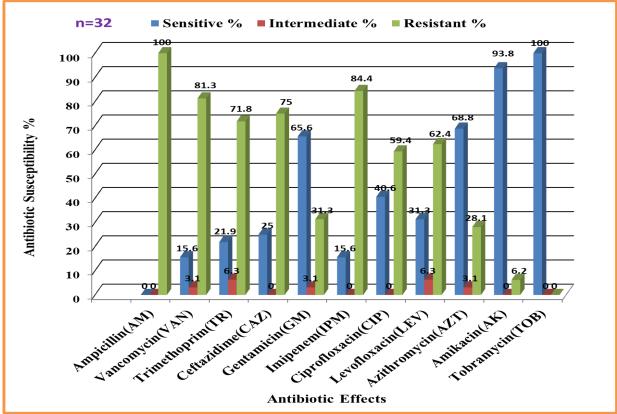
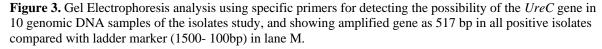
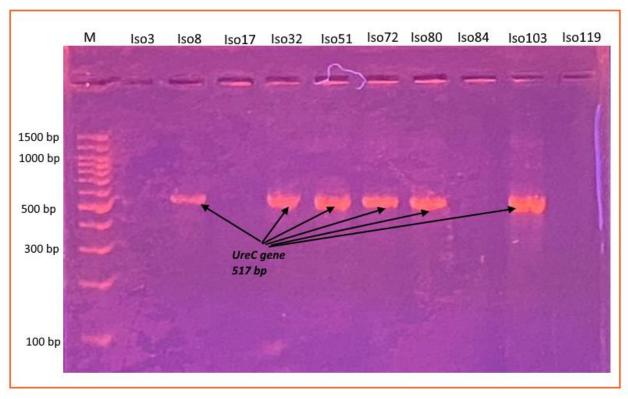


Figure 2. Chart showing effects of 11 Antibiotic Susceptibility Tests for *P. mirabilis* isolates.

Figure 2. Chart showing effects of 11 Antibiotic Susceptibility Tests for *P. mirabilis* isolates





#### Discussion

From 135 urine samples of clinical cases, 32 positive results of P. mirabilis were recorded, which constituted a high percentage (23.7%) compared with other studies. In a similar study, Gomaa et al.[11] found P. mirabilis in 15.2%, while Armbruster et al.[9] recorded 22% of this bacteria among other bacterial isolates in urine samples. The presence of bacteria in urine samples is generally attributed to numerous virulence factors that are considered important in generating UTIs, such as the capacity to adhere, the synthesis of urease, and flagella [16]. Similar results were found in another study conducted in Baghdad by Al-Bassam and Al-Kazaz [17]. The isolation rate of P. mirabilis from urine samples was found to be higher than the 4% reported in a study conducted in India by Rajkumar et al. [19]. In contrast, research from Iraq, including studies by Mahdi & Al-Deresawi [15] and Jarjes [12], reported similar rates of isolation.

These results are consistent with other research, which confirmed that P. mirabilis has a high level of ampicillin resistance, as Hussein et al. [16], Al-Bassam & Al-Kazaz [17], and Bahashwan & El Shafey [18] from Iraq. Consequently useless in treating illnesses brought on by this specific bacterial strain. In the isolated P. mirabilis, a high resistance to Imipenem may be caused by the loss of porins, the downregulation of the expression of the penicillin-binding proteins (PBPs), such as PBP1a and PBP2, or the accretion of other antibiotic resistance genes, which include Carbapenemase genes [19,20]. Patients with renal failure in Tikrit city had a significant rate of imipenem resistance, according to Al-Jebouri and Al-Alwani [21]. Furthermore, Tabatabaei et al., [22] found in their research on catheter-associated patients' UTIs in Iran, they verified that P. mirabilis isolated is distinguished as resistant to Imipenem. They ascribed this to the potential for antibiotic misuse, which leads to appear emergence of multipleresistant bacteria in the community.

Aminoglycoside medications are used to treat a variety of Gram-negative bacteria [23]. Iranian researchers published similar results for resistance of Amikacin and Gentamicin by this bacterium, but Jabur *et al.* [24] from Babylon found identical results for gentamicin resistance 50%, and extremely different results for amikacin resistance, estimated 5%. Different bacterial species develop

resistance to aminoglycosides via a range of acquired and innate mechanisms [22, 21].

The results of this study likewise slightly deviate from the Rafalskiy et al., [25]. He discovered that the resistance to Netilmicin was 38.1%, whereas the resistance to Gentamicin was 25.3%. Chen et al. [26] similarly showed that the resistance to amikacin and gentamicin was 67.7% and 58%, respectively, but that the resistance to Netilmicin was 45.1%, which was in agreement with our findings. Gazel et al. [27] from Turkey reported lower levels of sensitivity (35% and 26%), while Hussein et al. [16] from Iraq, notes that a high estimated level of sensitivity for antibiotics was reported (69.8%). However, among the studied aminoglycosides, tobramycin showed the highest level of resistance, measuring 100%. This result was consistent with Jarjes et al. [12], who reported that 70% or less of the Tobramycin resistance pattern was found, in contrast to Bashir et al. [23] and Chen et al. [26], also Tobramycin resistance values were registered at 9.4% and 48%, respectively. Antibiotic susceptibility and the expression of WGS resistance genes for numerous antibiotic classes were impacted by certain mutations in the amino acid sequences UreC gene [28].

The overuse of antibiotics, the use of antibiotics in the presence of a bacterial or fungal pathogen without medical advice or laboratory testing, and the community's health culture are the primary reasons for the increasing rates of bacterial resistance to antibiotics among individuals in different communities or within a single community.

Proteus mirabilis is capable of producing several virulence factors that are essential for human infection. The genetic complex involved in urease production consists of five helper genes: UreD, UreE, UreF, UreG, and UreR. Additionally, there are three structural genes—UreA, UreB, and UreC [29]. Each component forms a trimer complex within the urease enzyme, which is referred to as an apoenzyme. For the urease enzyme to function properly, nickel ions must be present in the metal core of UreC. According to Coker et al. [30], support proteins, such as the gene UreR, play a critical role in facilitating the integration of nickel into the active site of the urease enzyme, thereby synchronising its activation.

When employing the PCR technique for the amplification of DNA fragments of *P. mirabilis*, some unfavorable results were observed for UTI patients, such as DNA of (Iso3, Iso17, Iso84, and Iso 119 (Figure 3) and others. These results were consistent with what was reached by both Wang *et al.*, [31] and Dattelbaum *et al.*, [32]. This is attributed to the gene expression of the *UreC* gene; it is not related to any gene group or any other factors.

Recently, there has been increasing interest in studying many of the genes responsible for antibiotic resistance in many bacterial species, such as [33,34], intending to find ways to determine the effect of the antibiotic resistance or find more effective alternatives for pathogenic bacteria.

#### Limitations of the study

Several limitations in this study can be summarized as follows,

- 1- Samples of the study were selected from Patients living in a small governorate, but they represent different natives with social ties between them, making the sample potentially representative of larger Iraqi communities.
- 2-The study revealed a high level of resistance of *Proteus mirabilis* isolated from patients to most antibiotics, suggesting overuse of these antibiotics or the prescription of high doses in the hope of a speedy recovery, especially for patients with UTI.
- 3- The absence of the *UreC* gene in the DNA of bacterial isolates necessitates further studies on larger samples to investigate structural genes and other urease gene complexes.
- 4-The presence of the *UreC* gene in the bacterial isolates was not limited to an age group or linked to gender, but rather yielded diverse results in the study sample.

Based on the data and preliminary results of this study, more studies can be conducted on a larger number of clinical samples of UTI patients, including studies of other structural genes of urease, studding other bacterial genus or species related with UTI Disease se as well as studies of the relationship between types of stones and other salts crystals in the urine and the organs of the urinary system, which are formed in patients with urinary tract infections.

#### Conclusion

Studies indicate that urease expression is inhibited when a *UreC*-regulatory protein is absent.

This protein may represent a promising target for treating *P. mirabilis* infections as an alternative to antibiotics to which these bacteria have developed resistance. The current study indicates that while *P.mirabilis* isolates are primarily linked to urinary tract infections, the urease enzyme produced by pathogenic isolates plays a significant role in regulating intestinal pH. This finding was supported by examining the presence of the UreC gene, which encodes a large subunit responsible for both the production and function of the urease enzyme in some *P. mirabilis* isolates. Consequently, the results of the urease enzyme screening tests in pathogenic *P. mirabilis* isolates may not always yield a positive result.

#### **Conflict of Interest**

The authors declare that there are no conflicts of interest in this research.

#### **Authors' Declaration**

The authors declare that the research steps presented in this article were conducted under scientific controls, that the results obtained are original, and that they accept full responsibility for any claims related to the content of this article.

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