# Molecular Detection of (*Urec , Mrpa , Hpma*) Genes in *Proteus mirabilis* Bacteria Isolated From Patients with Urinary Tract Infection

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## ABSTRACT

**Background:** Urinary tract infection (UTI) is the term for bacterial colonization or infection of the urinary bladder, ureters and tract. **Objective:** This study aimed to detect specific virulence genes in *Proteus mirabilis* isolated from urine samples of Iraqi patients with urinary tract infection.

**Patients and methods:** During the study period from early December 2021 to late April 2022, urine specimens (103 specimens) were collected from patients with symptoms and signs of UTI from various hospitals in Baghdad and private laboratories in Baghdad. Samples were collected from men and women of different age groups (4-75 years) with clinically suspected UTI.

**Results:** All specimens were identified based on their properties on media (blood agar and MacConkey agar). Colonies on blood agar were identified as *P.mirabilis* due to their colonization, also had a characteristic fishy smell and appeared to form smooth, pale or colorless colonies on Macckongy agar instead, further diagnosed by using VITEK 2 system and molecular diagnosis by *urec* gene also gave positive results for 22 isolates of *P. mirabilis*. (*mrpA, hpmA*) genes detection results: *mrpA* presence percentage was 100%, while *hpmA* was 22.72%.

**Conclusion:** The molecular technique showed that the *mrpA*, *hpmA* and *urec* genes associated with virulence factor include: adherence activity, produce Alpha (α) hemolysin and urease.

**Keywords:** *Proteus mirabilis***,** Urinary tract infections, PCR, Gene.

## INTRODUCTION

Urinary tract infections (UTIs) occur when harmful microscopic organisms enter your urinary tract, most UTIs are caused by bacteria. This condition can affect the urinary tract, which is referred to as urethritis; the kidneys, which is referred to as pyelonephritis; and the urinary bladder, which is referred to as cystitis [(1)]. *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis* and *Enterobacter cloacae* are the most common bacterial UTIs [(2)].

Risk factors of UTIs are female anatomy, sexual activity, diabetes, family history, and obesity [(3)]. Some groups of people are more prone to UTI than others, for example diabetic patients and female. Females have fourteen times more chance to develop UTI than men due to several factors. Such as they has a shorter urethra compare to men, which open nearer to the anus so that the lower third of urethra is continually contaminated with pathogens from vagina and rectum [(1)].

Uncomplicated UTIs usually affect the bladder only. Cystitis occurs when bacteria invade the lining of the bladder. Most organisms that cause UTIs are enteric coliforms that normally inhabit the vaginal opening around the urethra. These microorganisms travel through the urethra into the bladder and cause UTIs. People who urinate frequently and void their bladders have a lower risk of UTIs [(4)].

This study aimed to detect specific virulence genes in *Proteus mirabilis* isolated from urine samples of Iraqi patients with urinary tract infection.

## MATERIALS AND METHODS

### 1. Samples collection:

In this study, 103 urine specimens were collected from patients with signs and symptoms of urinary tract infection from different age groups (4-75 years) of both sex in different hospitals and private laboratories in Baghdad from the beginning of December 2021 to the end of April 2022.

### 2. Isolation and identification of *P. mirabilis*:

In the laboratory, under sterile conditions, all urine samples (103) were cultured on (blood, nutrient agar, and MacConkey agar) at 37 °C for 24-48 hours, and further diagnosis was made for suspected colonies based on morphological characteristics and biochemical tests, and the molecular approach of the *ureC* gene with specific primers.

### Molecular methods

### Genomic DNA extraction

DNA extraction was performed; *Proteus mirabilis* isolates were cultured in brain heart broth for 24 hours at 37°C. Genomic DNA was isolated from bacterial growth according to the Favour Prep Blood/Cultured Cell Genomic DNA Extraction Mini Kit protocol. A Quantus Fluorometer is used to determine the concentration of extracted DNA to determine sample quality for



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downstream applications. Specific primers were used to detect *P. mirabilis* and some virulence genes (**Table 1**), according to PCR program for genes amplification **table 2**.

**Table 1**: **Primer sequences and sizes used to detect certain virulence genes**.

| Genes | primer Sequences (5→3) | Size (bp) |
|---|---|---|
| *UreC* | F: CCG GAA CAG AAG TTG TCG CTG GA<br>R: GGG CTC TCC TAC CGA CTT GAT C | 533 [5] |
| *MrpA* | F: TTC TTA CTG ATA AGA CAT TG<br>R: ATT TCA GGA AAC AAA AGA TG | 565 [6] |
| *HpmA* | F: GTT GAG GGG CGT TAT CAA GAG TC<br>R: GAT AAC TGT TTT GCC CTT TTG TGC | 709 [7] |

**Table 2: PCR program for genes amplification.**

| **Steps** | **Cycles** | **Genes amplification conditions** | | |
|---|---|---|---|---|
| | | ***UreC*** | ***HpmA*** | ***mrpA*** |
| **Initial denaturation** | **1 cycle** | **94 ºC (3 min.)** | **95 ºC (5 min.)** | **94 ºC (3 min.)** |
| **Denaturation** | **40 cycles (*UreC*)<br>35 cycles (*HpmA*)<br>30 cycles (*mrpA*)** | **94 ºC (1 min.)** | **95 ºC (30 sec.)** | **94 ºC (30 sec.)** |
| **Annealing** | | **63 ºC (30 sec.)** | **62 ºC (30 sec.)** | **40 ºC (30 sec.)** |
| **Extension** | | **72 ºC (1 min.)** | **72 ºC (20 sec.)** | **72 ºC (30 sec.)** |
| **Final extension** | **1 cycle** | **72 ºC (7 min.)** | **72 ºC (5 min.)** | **72 ºC (7 min.)** |

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**Ethical consent:**

**The study was conducted under the approval of the Scientific Research Ethics Committee of the Institute of Genetic Engineering and Biotechnology, Graduate School, University of Baghdad. Informed consent was obtained from the patient's relatives or the patient himself when the patient was aware of the need to keep patient records confidential at all stages of the study. This work was performed in accordance with the World Medical Association Code of Ethics for Human Research (Declaration of Helsinki).**

## RESULTS

**Traditional and biochemical methods:**

The isolation and identification of *Proteus mirabilis* is mainly based on the morphological characteristics of its culture traits and biochemical characteristics. A total of 103 different urine specimens were cultured on various media. These included blood, MacConkey and nutrient agar plates. The isolates obtained from these media were identified by their shape characteristics, that is, colonies on blood agar were identified as *P. mirabilis* by their clustering phenomenon, and by their characteristic fishy Growth in continuous waves forming concentric thin film layers (swarms), on Macckongy agar the colonies do not appear to form colonies but are smooth, pale or colourless colonies [8], which give positive results for 22 isolates *P. mirabilis* , then diagnosed by the VITEK 2 system and also gave positive results for 22 isolates o *P. mirabilis.*

**Molecular methods**

**Genomic DNA extraction:**

Genomic DNA was extracted from 22 bacterial isolates diagnosed by the VITEK 2 system. Total DNA was extracted from cultured cells using the Favor-Prep Blood/Genomic DNA Extraction Kit, and Quantiflour was used to measure the concentration and purity of the extracted DNA. The DNA concentrations of all isolates were in the range of 62.1-12.5 ng/µL, and the purity is in the range of 1.8 -1.98. Thereafter, the gel electrophoresis detection has been shown in **Figure 1**.

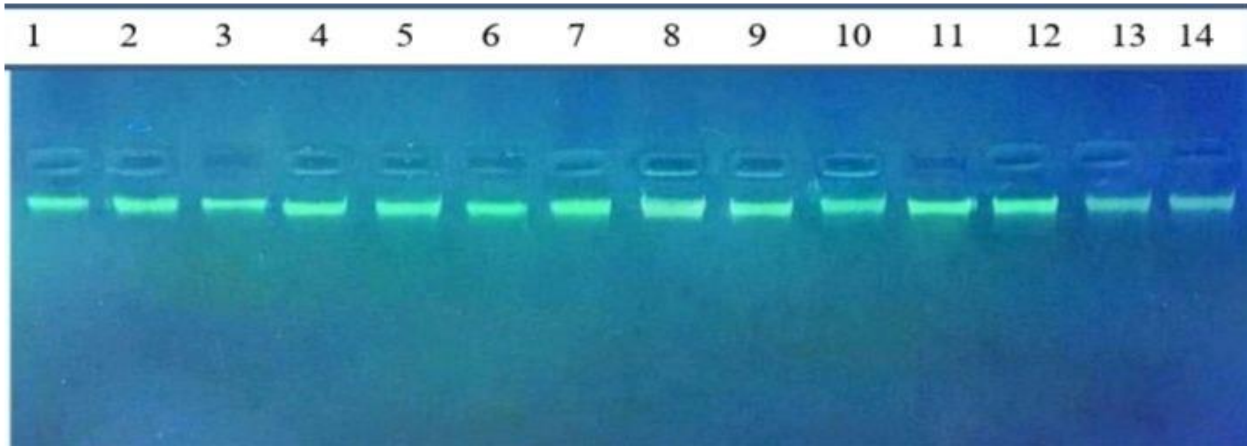


**Figure 1:** Genomic DNA extraction by electrophoresis (1% agarose gel, 75 V, 30 min).

To identify all 22 isolates of *P. mirabilis*, specific primers for the *urec* gene were used according to the purpose, which gave the same results compared to biochemical tests and the vitek2 system. Gel electrophoresis detection of all products of 22 conventional PCR is shown in **Figure 2**.

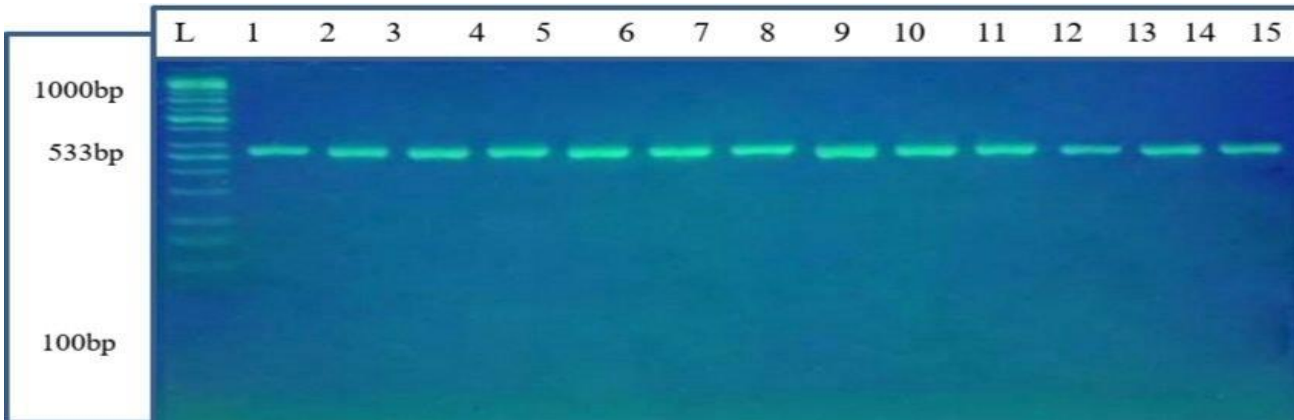


**Figure 2:** *UreC* gene (size 533 bp) extraction 75 volts 2% agarose gel electrophoresis for 1 hour. L: Ladder with 1000bp.

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**Detection of some virulence genes:**

**Table 3** show the results for the genes *hpmA* and *mrpA* by Conventional PCR, which are both found in *P. mirabilis*. Results from this process were detected using gel electrophoresis. Additional information can be found in the same documents as well as in **Figures 3 and 4**.

**Table (3): Total number and percentage of *mrpA* and *hpmA* genes in *Proteus mirabilis***

| Genes | Total number | Percentage |
|---|---|---|
| *mrpA* | 22 | 100% |
| *hpmA* | 5 | 22.72% |

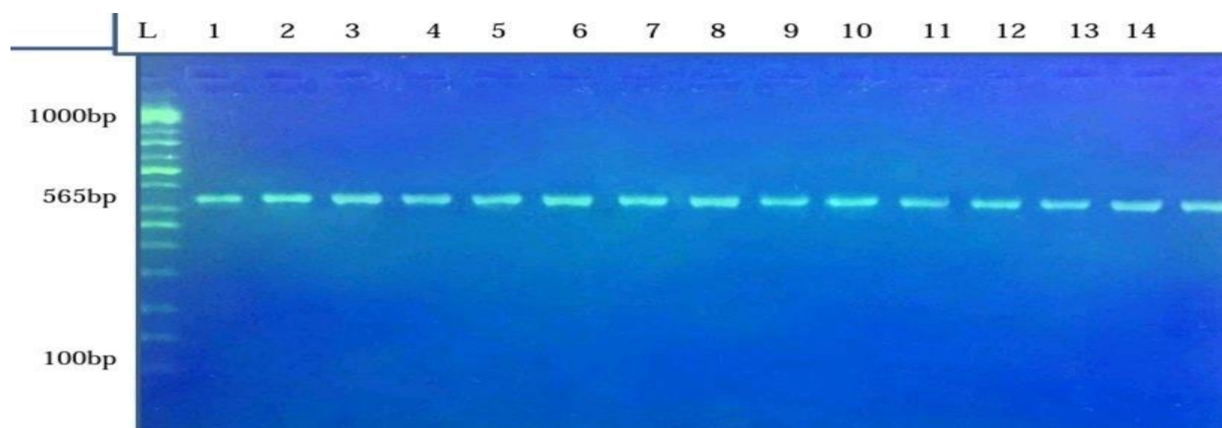


**Figure 3:** Agarose gel electrophoresis (2% agarose, 75 V, 1:45 hr) of the *mrpA* gene PCR product (565 bp in length). L: Ladder with 1000bp.

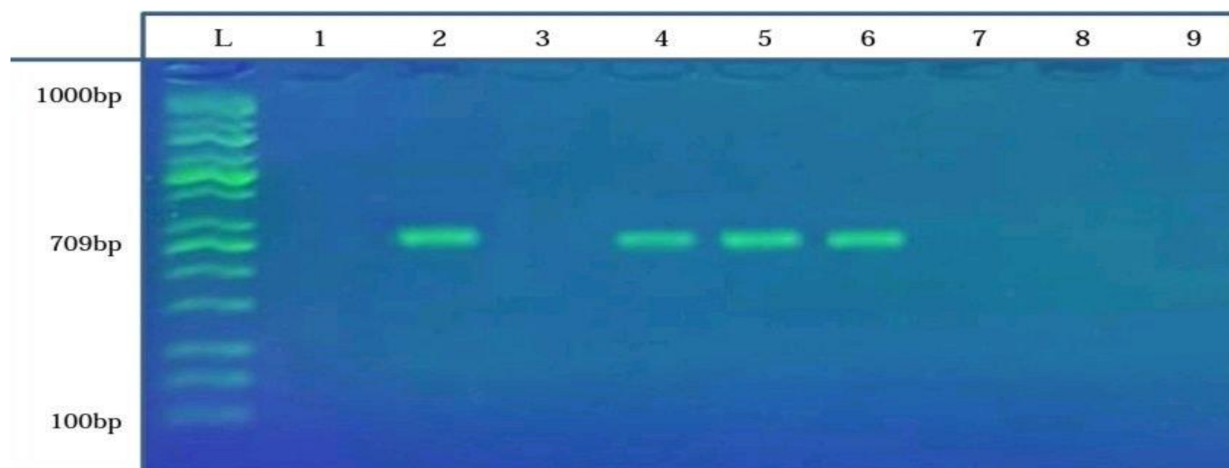


**Figure 4:** Agarose gel electrophoresis (2% agarose, 75 V, 1:45 hr) of *HpmA* gene-gene PCR product (length 709 bp). L: Ladder with 1000bp.

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## DISCUSSION

Twenty two isolates identified based on their properties on media (blood agar and MacConkey agar). Colonies on blood agar were identified as *P.mirabilis* due to their colonization, also had a characteristic fishy smell and appeared to form smooth, pale or colorless colonies on Macckongy agar instead, further diagnosed by using VITEK 2 system and molecular diagnosis by *urec* gene also gave positive results for 22 isolates of *P. mirabilis* and this results match with results of traditional and biochemical methods .

*Proteus mirabilis* has an essential gene related to the production of urease, which is the key enzyme in stone formation in both the kidney and bladder. This *ureC* gene is necessary for this process [9]. Metallic enzymes increase the pH in the body and result in crystals being formed.

The MR/P pili gene is important because it provides many virulence traits, such as colony formation, formation of biofilms and bacterial attachment. The important type of the MR/P pili gene is *mrpA, mrpB, mrpC, mrpD, mrpE, mrpF, mrpG* and *mrpI.* Each of these genes code for one type of MR/P pili.

Furthermore, the study showed that the *mrpA* gene was present in 100% of all 22 isolates with a molecular size of 565 bp. This study is consistent with a previous study [10].

Furthermore the investigation showed the presence of *HpmA* gene in 22.72 % of isolates with

molecular size of 709bp .The results presented here nearly match with the results of previous study that found *hpmA* gene was 30% [11]. The *hpmA* gene is a virulence factor that helps cause urinary tract infections. This gene is significant as it causes infections in other areas of the urinary tract.

*Proteus mirabilis* bacteria produce α-hemolysin, which causes damage to kidney tissue. This destructive protein is one of two genes, *hpmA* and *hpmB*, that control *HpmA*, which is a larger, calcium-independent protein with a N-terminal peptide (166 kDa). When α-hemolysin breaks through the pores in its calcium-independent form, the result may be activation of *HpmA* (140 kDa) [13].Increased *HpmA* production is coordinately regulated during cell differentiation into colony forms and infection and correlates with invasiveness of *P. mirabilis* strains [7].

## CONCLUSION

Based on the results of this study, the following conclusions can be made clear. Quick methods of detecting the *urec* gene using molecular tools can be found. Molecular engineering revealed that *mrpA, hpmA*, and *urec* genes associated with virulence factors include: adhesion activity, production of alpha (α) hemolysin, and urease.

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