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Research Article

## Molecular study of *hpmA* and *ureR* genes among *Proteus mirabilis* isolates inpatient with urinary tract infection

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### Abstract

The most frequently occurring human bacterial infections are urinary tract infections (UTIs). *P. mirabilis* is one of the common causes of UTIs in humans with complicated UTIs, the formation of stones in the kidney, and long-term indwelling catheters. This study aims to isolate, and identify *Proteus mirabilis* from patients with UTIs using traditional methods and 16s rRNA gene sequencing techniques, as well as detect some virulence genes (*hpmA* and *ureR*) in *Proteus mirabilis* by using polymerase chain reaction (PCR). A total of five isolates of *Proteus mirabilis* were isolated from patients with urinary tract infections in June 2022. Identification of isolates depends on microscopic and cultural characteristics and traditional biochemical tests, finally, isolates are identified by target gene 16s rRNA using PCR technique, in addition, this study investigates the genes responsible for virulence factors such as *hpmA* and *hpmR*. In this study laboratory diagnosis and molecular analysis (16s rRNA gene) revealed that the five isolates are *Proteus mirabilis* also this study shows the presence of the *hpmA* genes by PCR techniques in the isolates 100%. Our study has shown that 16s rRNA was a strong eclectic power and an excellent method for the identification of bacteria also these study frequency of *ureR* and *hpmA* in *P. mirabilis* isolated from UTIs and these genes have a manifest role in the infection.

**Keywords:** *Proteus mirabilis*, Virulence Genes, Urinary Tract Infection.

### Introduction

*Proteus* is connected to Enterobacteriaceae, is a gram-negative rod bacterium, facultatively anaerobic, unable to form spores, used for motility peritrichous flagella and adhesion used fimbriae (Murray et al., 1999) includes five species *Proteus mirabilis*, *Proteus hauseri*, *Proteus vulgaris*, *Proteus penneri* and *Proteus myxofaciens*, and *Proteus spp* may collaborate in infections of wounds, throat, gastrointestinal tract, bones, skin, eyes, respiratory tract, ears and urinary tract infection. Generally, *P. mirabilis* is found in the human intestine, where cohabits with other microflora and causes infections when leaves the intestinal tract. Also is found in the environment, and used disinfectants to keep them out of hospitals (Al-Mudallal et al., 2021). UTI infections with *P. mirabilis* may induce a variety of complications such as fever, bacteremia,

renal stones, acute pyelonephritis, catheter obstruction, bacteriuria and chronic inflammatory changes (Rezatofighi et al., 2021; Sosa and Zunino, 2010). Potential virulence factors and bacterial behaviors associated with the disease, including flagella, swarming, growth rates, production of urease, hemolysins amino acid deaminases, proteases and expression of lipopolysaccharide antigens and capsule polysaccharide (Al-Dawah et al., 2015).

*P. mirabilis* produced hemolysin *HpmA* that associated to the cell, calcium-independent, former of pores and encoded by two genes includes *hpmA* and *hpmB*, that regulate the *HpmA* protein. Urease is a major virulence factor for *P. mirabilis* it is generate ammonia from the hydrolysis of urea present in urine this lead to generate carbonate-apatite kidney or bladder stones (Rezatofighi et al., 2021; Kearns, 2010). *UreR* is activate expression of the urease operon, there is no induction of

urease expression in the absence of urea (Armbruster et al., 2018).

This study aims to isolate and identify *P.mirabilis* from patients using traditional methods and 16s rRNA gene sequencing techniques, as well as detecting some virulence genes (*hpm A* and *ure R*) in *Proteus mirabilis* by using polymerase chain reaction (PCR).

## Materials and methods

### Bacterial Strains and Identification

Five isolates of bacteria *P. mirabilis* were used in this study. Isolates were obtained from patients with UTIs in June 2022 (from the microbiology laboratory of Tikrit Military Hospital, Tikrit, Iraq). MacConkey agar, nutrient agar, and blood agar were used for the isolation of *P.mirabilis*. Cultures were

incubated aerobically at 37<sup>o</sup> C. The laboratory diagnosis of isolates was based on morphology features, microscopic examination, and biochemical tests then diagnosis was completed with PCR technique by using target gene 16s rRNA.

### Extraction of DNA and PCR

#### DNA extraction

The DNA of five *P.mirabilis* isolates was extracted as described by the instruction of the Promega kit.

#### Preparation the primers

Primers used in this study are shown in Table 1.

**Table 1. Sequence of primers of *E. coli* genes**

Target gene	Primer sequence	Size of amplicon (bp)	References
<i>ure R</i>	F-GCGGTTTATCACGAAGGGGT R- TGAGTGCGAAATTGCGATGG	359	(Dattelbaum et al.,2003)
<i>hpmA</i>	F-GTTGAGGGGCGTTATCAAGAGTC R-GATAACTGTTTTGCCCTTTTGTGC	709	(Cestari et al., 2013)
<i>16S rRNA</i>	F:TGAAGAGTTTGATCATGGCTCAG R:ACCGCGGCTGCTGGCAC	527	(Park et al., 2011)

### PCR amplification

PCR amplification was done for extracted DNA of bacteria in a total volume of 25 ul containing 5 ul template DNA, 0.5 ul of each of the primers, nuclease-free water 6.5 ul, and master mix 12.5 ul.

X1: annealing temperature for each primer of *Proteus mirabilis* virulence genes; ure R (55 C), hpm (55 C) and 16s (58 C).

X2: annealing time for each primer; ure R (50s), hpm and 16s (1min).

PCR products were separated on 1.5 % agarose gel containing ethidium bromide and visualized under UV light, then photographed by digital camera.

**Table 2. The PCR thermos cyler program**

Stage	Temperature	Time	Number of cycles
Initial denaturation	95	5 min	1
Denaturation	95	40 s	35
Annealing	X1	X2	
Extension	72	40 s	
Final extension	72	7 min	1

### Result and discussion

The results show the growth of five isolated *P. mirabilis* on the surface of nutrient agar and blood agar with a swarming

phenomenon, colonies on MacConkey agar appear pale and non-lactose fermenters. The microscopic examination n showed a gram-negative bacteria rod shape. The biochemical tests were done according to (Brown and Smith, 2015).

The advantage of manual procedures was inexpensive. The identification was completed by using the PCR technique using 16s rRNA and the result had a profile that was shown by intense bands at 527 in the 16s rRNA gene PCRs, all isolates were *P.mirabilis* strains as shown in the figure. This study matches the results of Jaber and AL. miyah (2022) when they used 16S rRNA to identify the isolates of *P.mirabilis*. About 90% of proteus infections is caused by *P.mirabilis* (Jones et al., 1990). Detection of bacteria by 16s rRNA genes acts as an exact tool for the identification of bacteria in hospitals and environments, also considered a good routine and a very useful method in microbial communities. 16s rRNA gene is strongly preserved within species and among species of the same genus, so, can be used as an alternative technique for the identification of bacteria at the species level (Mukhtaret al., 2018).

The results of the current study have shown that *hpmA* genes were present in isolates of *P.mirabilis* by the intense band at 709 in the *hpmA* genes PCR and at a rate (100%) as shown in

Fig 2. This result matches the results recorded by Ali and Yousif (2015) they mentioned that the rate of *hpmA* gene in *P. mirabilis* isolates is %100 in patients with UTI infection. These results also match the result recorded by Lazm et al., (2018) mentioned that the rate of the gene *hmpA* of *P.mirabilis* isolated from UTIs is 100%, while Ghaima et al., (2019) found the ratio of gene *hpmA* and *hpmB* in bacterial isolates 87.3 %. This current study means that hemolysin increases the virulence of *Proteus mirabilis* to causing infections in humans such as UTIs infections, protein has been related with cytotoxicity in vero cells because production of this also observed that *P.mirabilis* which produced hemolysin related to cell produced a lethal dose 50% better than non hemolytic *P.mirabilis* when inoculated in mice (Rozalski et al., 1997). The hemolysin production lead to destroy the kidney tissue of the host by its cytotoxic effects on the leukocyte membrane and epithelial cell (Liaw et al., 2003), it's capable of lysing bladder epithelial cell due to the a combination of the hemolysin and toxic agglutinin (Armbruster et al., 2018).

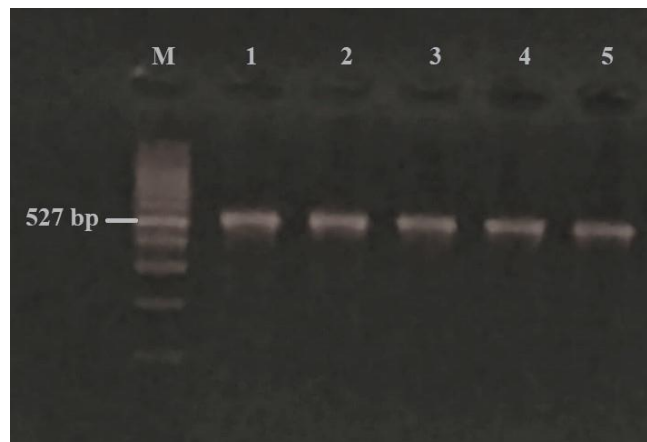


Fig. 1 Amplification of 16S rRNA genes of *P. mirabilis* by PCR. Lanes: M, 100 bp ladder marker, (1-5) clinical isolates were positive results for 16S rRNA genes for *P. mirabilis*

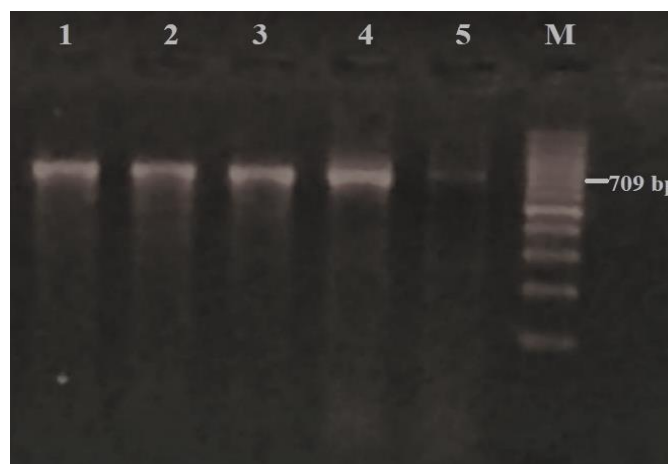
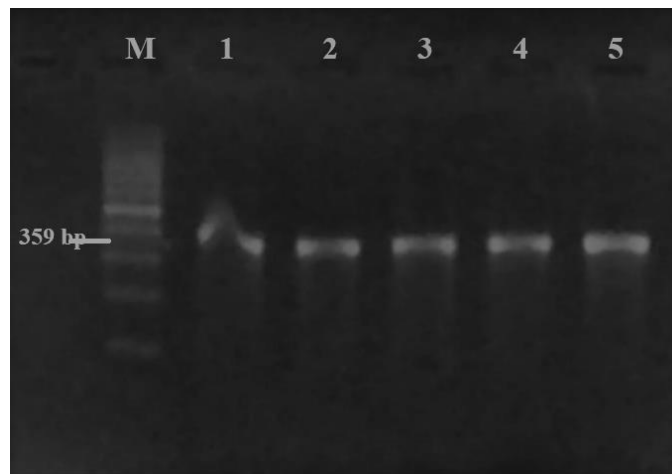


Fig. 2 Amplification of *hpmA* genes of *P.mirabilis* by PCR. Lanes: M, 100 bp ladder marker, (1-5) clinical isolates were positive results for *hpmA* genes for *P.mirabilis*

The results of this study also showed that all the isolates have *ureR* gene as shown in Fig. 3 had a profile that was shown by intense bands at 359 in the *ureR* gene PCR sand the rate of 100% this result agrees with Jaber and ALmiyah (2022) which found this gene in *P.mirabilis* at rate 100% .The *ureR*gene in *P.mirabilis* is specialized gene in diagnosis of *P.mirabilis*and is responsible for production of urease enzymethat is amajor

virulence factor in *P.mirabilis*, urease catalyzes the hydrolysis of urea to carbon dioxide and ammonia therefore the pH of urineincreases , magnesium phosphates and calcium begin to precipitate out of solution leading to the formation of crystals (calcium phosphate and magnesium ammonium phosphate).Urease activity strongly related with renal tubule damage and nephrosis during UTI (Armbruster et al., 2018) .



**Fig. 3 Amplification of *ureR* gene of *P.mirabilis* by PCR. Lanes: M, 100 bp ladder marker, (1-5) clinical isolates were positive results for *ureR* genes for *P.mirabilis***

## Conclusion

Our study has shown that 16s rRNA was a strong eclectic power and an excellent method for identification of bacteria. Also these study frequency of *ureR* and *hpmA* genes in *P.mirabilis* isolated from UTIs and these genes have a manifest role in the infection.

## Conflict of Interest

The author hereby declares no conflict of interest.

## Consent for publication

The author declares that the work has consent for publication.

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