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Analysis of UreC, MrpA, HpmA, and UreR Genes in *Proteus mirabilis* and Their Single Nucleotide Polymorphisms

Hussein A. Naser, Basil A. Abbas*, Ali B. Al-Deewan
Department of Microbiology, College of veterinary medicine, University of Basrah, Iraq
*Correspondence author: basil.abbas@uobasrah.edu.iq

Abstract

Proteus mirabilis is a Gram-negative opportunistic pathogen commonly implicated in urinary tract infections (UTIs) in both humans and animals. This study aimed to isolate and identify *P. mirabilis* from urine samples of humans, cats, and dogs, and to evaluate the presence and genetic variability of selected virulence genes. A total of 150 urine samples (50 from each host species) were collected using sterile techniques. Isolation was performed on HiCrome UTI agar, and identification was confirmed using the VITEK-2 system. PCR was employed to detect four virulence genes: ureC, hpmA, mrpA, and ureR, followed by sequencing and SNP analysis. Out of the 150 samples, 18 isolates were identified as *P. mirabilis*. DNA sequencing revealed varying numbers of SNPs among the genes, with mrpA exhibiting the highest polymorphism, suggesting potential genetic diversity and adaptation mechanisms. These findings highlight the zoonotic potential of *P. mirabilis* and emphasize the importance of molecular characterization to understand its virulence and evolutionary dynamics in different hosts.

Key words: UreC; MrpA; HpmA; UreR; *Proteus mirabilis*

Introduction

Proteus mirabilis is a Gram-negative bacterium belonging to the *Enterobacteriaceae* family and is a leading cause of urinary tract infections (UTIs), particularly in patients with long-term catheterization [1; 2]. This bacterium exhibits remarkable adaptability to the urinary environment due to a variety of virulence factors, including proteolytic enzymes, adhesion factors, exotoxins, and regulatory systems that enhance its survival and colonization [3]. Among the most crucial

genes involved in its pathogenicity are UreC, MrpA, HpmA, and UreR, which contribute to infection establishment, biofilm formation, resistance to adverse conditions, and host tissue damage, ultimately exacerbating bacterial persistence and disease severity [4; 5]. The UreC gene is a key determinant in the production of urease, an enzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide. This mechanism underscores the role of *UreC* in *P. mirabilis* pathogenicity and explains why UTIs caused by this bacterium are often difficult to eradicate [6]. The UreR gene serves as a regulatory element that activates the transcription of urease-related genes, including *UreC*, in response to urea availability in the surrounding environment. *UreR* functions as an activator protein that binds to the urease operon promoter, ensuring that urease production is tightly controlled and only induced when necessary [7].

MrpA plays a pivotal role in bacterial adhesion to urothelial cells. It encodes a structural subunit of the MR/P fimbriae, a type of pili that facilitates bacterial attachment to host surfaces, promotes biofilm formation, and provides resistance to shear forces within the urinary tract [8]. Adhesion is the first step in bacterial colonization, enabling *P. mirabilis* to evade the flushing action of urine, a key host defense mechanism that removes pathogens from the bladder. Studies have shown that disruption of *MrpA* results in a significant reduction in bacterial adherence, indicating its essential role in *P. mirabilis* colonization and persistence [9].

HpmA is a gene encoding the hemolysin toxin, an exotoxin that aids *P. mirabilis* in acquiring iron, a crucial element for bacterial growth and survival within the host. Iron is a limited resource in the human body, as it is sequestered by transport proteins such as lactoferrin and ferritin. Furthermore, studies suggest that *HpmA* enhances the ability of *P. mirabilis* to ascend from the bladder to the kidneys, increasing the risk of acute pyelonephritis, a severe kidney infection that can lead to bacteremia and systemic complications [10; 11].

Materials and Method

Sample collection and bacterial isolation

The samples were collected from human and domestic pets (cats and dogs). The samples were then examined in the laboratory. "Samples were cultured them on Crome UTI agar, a medium specifically designed for the growth of *Proteus mirabilis*. Once colonies appeared on the plate, we purified the colony by taking a single, pure isolate and cultured it on MacConkey agar. Upon observing the colony, we identified it based on its shape, color, and distinctive odor, which is a characteristic feature of this bacterium.

Finally, the diagnosis was confirmed by the using the VITEK system.

Molecular diagnosis:

The following primers were used for PCR.

Ure C; F: 5'-CCG-GAA-CAG-AAG-TTG-TCG-GCT-GGA-3' ; R:5'-GGG-CTC-TCC-TAC-CAG-CTT-GAT-C-3'; ***MrpA*** *F:* 5'-TTC-TTA-CTG-ATA-AGA-CAT-TG-3' ; *R:* 5'-ATT-TCA-GGA-AAC-AAA-AGA-TG-3'

HpmA *F:*5'-GTT-GAG-GGG-CGT-TAT-CAA-GAG-TC-3' R; 5'-GAT-AAC-TGT-TTT-GCC-CTT-TTG-TGC-3'

HpmA *F:*5'-GTT-GAG-GGG-CGT-TAT-CAA-GAG-TC-3' ; R;5'-GAT-AAC-TGT-TTT-GCC-CTT-TTG-TGC-3'

Result

Bacterial culture

The samples were then cultured on a special agar plate called HiCrome UTI Agar and incubated at 37°C for 24 hours. After incubation, colonies appeared on the agar (Figure 1).

Molecular study

PCR of *ureC* gene

All isolated bacteria also subjected to detect the presence of specific gene (*ureC*). All isolates show presence of this gene indicates and confirmed that this bacterium is *Proteus merabilis* (Fig. 2).

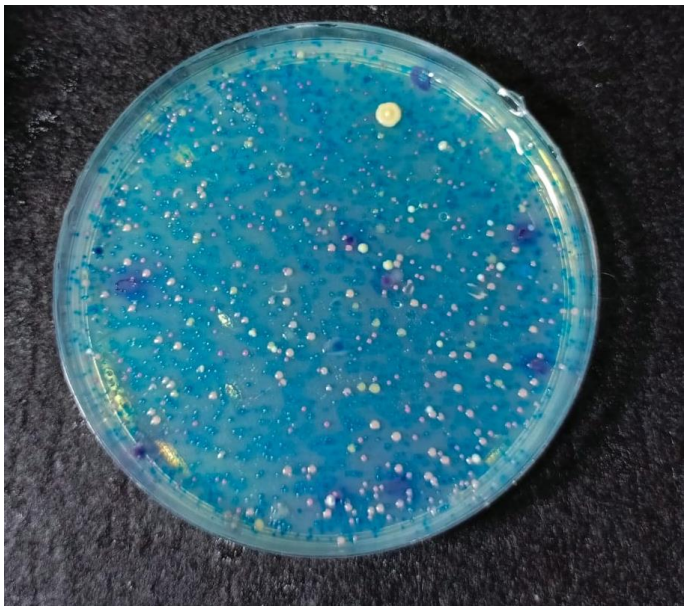

Figure 1. ***Proteus mirabilis*** **(white colonies) on HiCrome UTI Agar.**

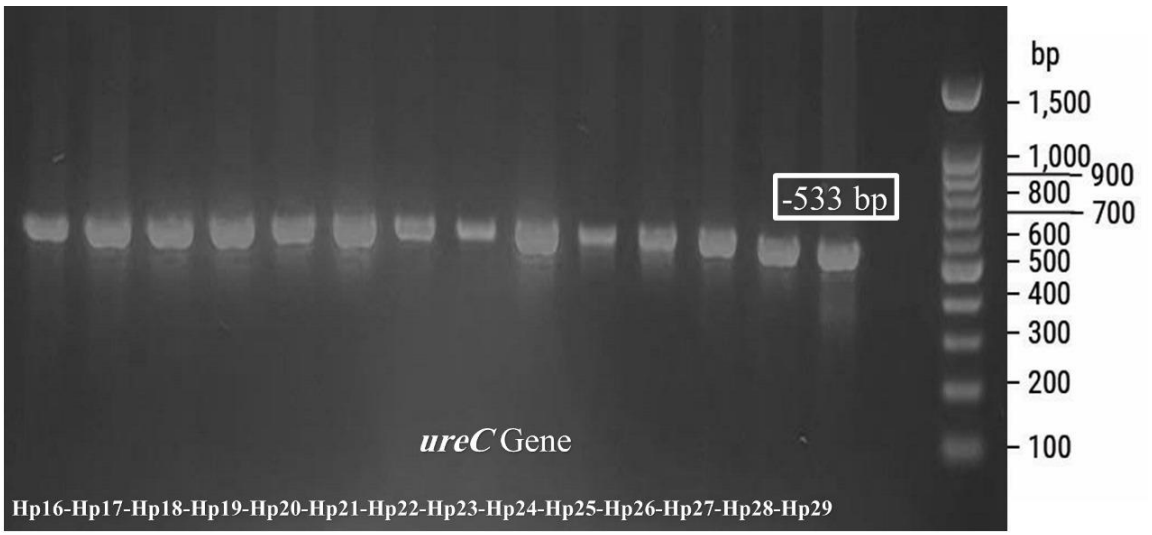

Figure 2. PCR of ureC gene of *Proteus mirabilis* isolated from cat and human, showing 533bp results using 2% agarose gel electrophoresis.

Detection of Ure Genes

Three *ure* genes were detected in this study, *hpma, mrpa and ureR* (fig. 3, 4, 5). The results showed the presence of all genes in all isolated bacteria.

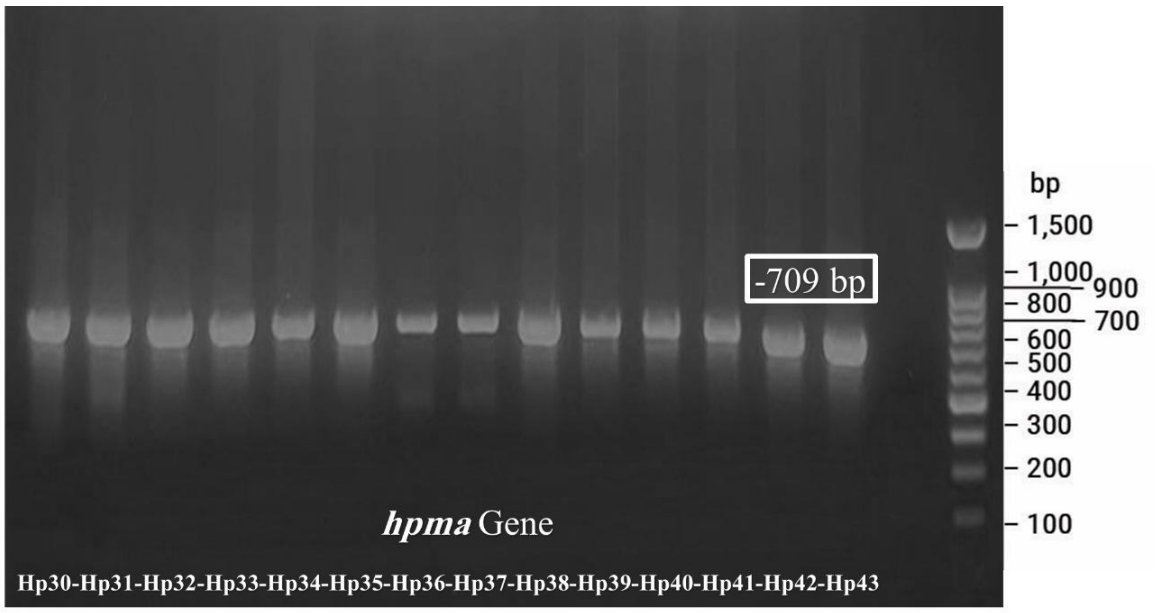

Fig.3. PCR of *hpmA* gene of *Proteus mirabilis* isolated from cat and human, showing 709bp results using 2% agarose gel electrophoresis.

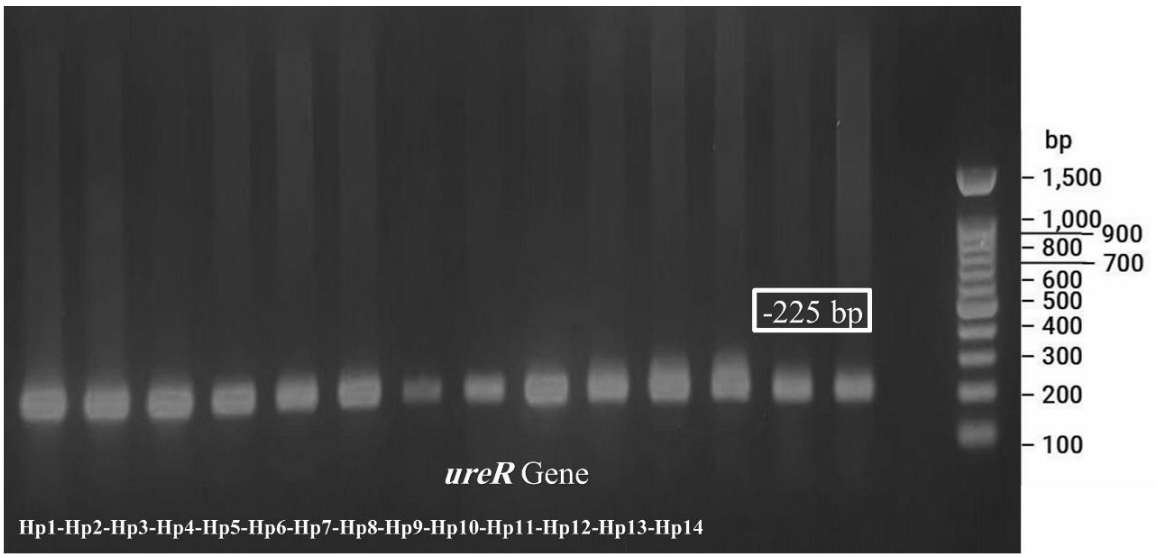

Fig.4. PCR of *ureR* gene of *Proteus mirabilis* isolated from cat and human, showing 225bp results using 2% agarose gel electrophoresis.

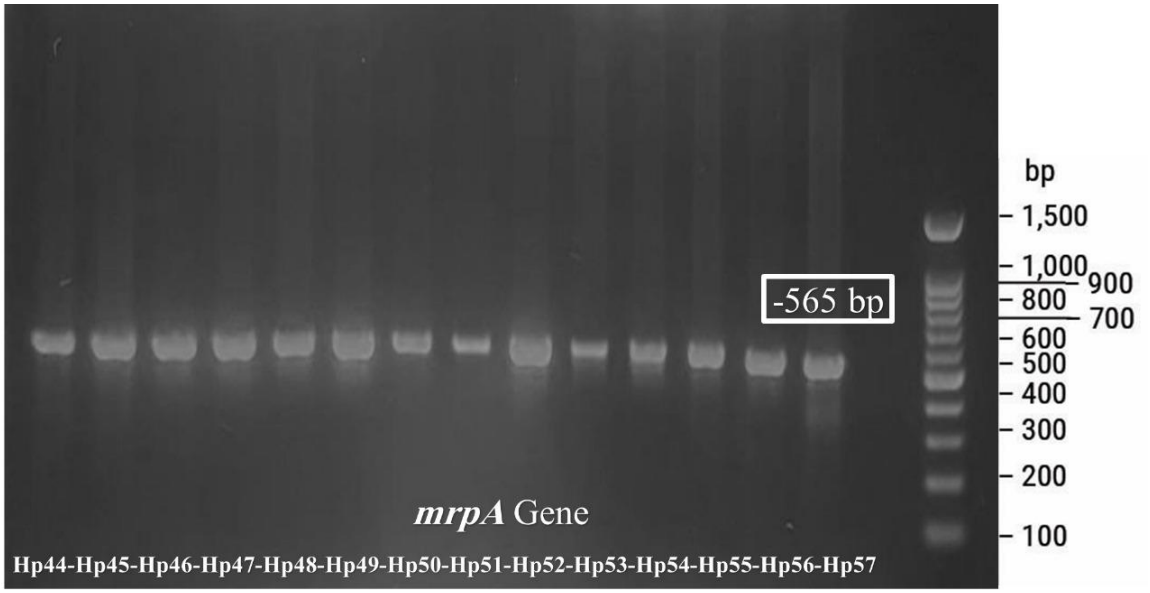

Fig. 5. PCR of *mrpA* gene of *Proteus mirabilis* isolated from cat and human, showing 565bp results using 2% agarose gel electrophoresis.

DNA sequencing of the genes:

Sequencing of ure genes reveals multiple SNP in genome in comparison with a reference sequence ID: CP189757.1. Table 1 summarize the total differences between the foe all genes.

Table 1 . Single nucleotide polymorphism of Ure genes in compared to sequence ID: CP189757.1

Gene	No of sequence	Compared ID	Change No.	SNP
P31_HpmAF	11-682 Range: 2826773 to 2827444	CP189757.1	5	-/A; G/A; G/A; G/-; G/-
HP45_MrpAF	10-522 Range: 886035 to 886548	CP189757.1	2	-/T; A/T
HP29_UreCF	1-453 Range: 552485 to 552937	CP189757.1	3	A/G; A/G; A/G
HP2_ureRF	11-197 Range: 549815 to 550001	CP189757.1	2	T/C; A/T

Discussion

At the molecular level, polymerase chain reaction (PCR) was used to detect four main virulence-associated genes in *P. mirabilis* isolates: *ureC*, *ureR*, *mrpA*, and *hpmA*. All isolates tested positive for these genes, which reinforces the phenotypic diagnostic findings and confirms the pathogenic nature of these strains. The presence of *ureC* and *ureR* is particularly important due to their crucial role in the regulation and production of urease, a major factor in *P. mirabilis* physiology. Urease degrades urea into ammonia and carbon dioxide, increasing urine pH and promoting struvite crystal formation. Mobley et al. [3] confirmed that urease not only raises urine pH but also plays a pivotal role in the pathogenesis of chronic and complicated UTIs. Furthermore, sequencing of the four virulence genes was conducted using modern sequencing technologies, and the results were compared with reference genome sequences in the NCBI database (reference strain CP070569.1). This analysis revealed the presence of single nucleotide polymorphisms (SNPs), especially in *mrpA* and *hpmA*. The *mrpA* gene is responsible for producing

specific fimbrial proteins involved in urinary tract adhesion, while *hpmA* encodes hemolysin, a cytotoxin that damages host tissues. These SNPs indicate significant genetic diversity between Basrah isolates and reference strains, supporting the hypothesis of locally adapted strains. Guentzel [12] noted that mutations in virulence genes, particularly those related to adhesion and secretion, can directly affect the severity and persistence of UTIs by altering protein effectiveness. Additionally, in-depth sequencing of the *ureC* gene revealed both conservative and non-conservative mutations across different sites. These mutations affect the structure and function of the resulting urease enzyme. Non-conservative mutations, in particular, may alter critical amino acids within the enzyme's active site, enhancing or diminishing its activity. This directly influences the bacterium's ability to increase urine pH and form urinary stones. Poore and Mobley [13] supported this observation, showing that urease gene alterations are directly related to the ability of bacterial isolates to induce environmental changes that promote stone formation, especially in recurrent or chronic infections. Several previous studies deals with bacteria other than *P. mirabilis* in Basrah city [14-17]. The presence of these mutations in *P. mirabilis* isolates from Basrah may serve as molecular evidence of local adaptation and evolutionary development, potentially explaining their pathogenic behavior in different hosts (humans, cats, and dogs). These findings call for further molecular and immunological studies to understand the impact of these mutations on host-pathogen interactions.

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تحليل جينات

UreR وHpmA وMrpA وUreC

في البروتيس وتعدد اشكالها النيوكليوتيدية المفردة

حسين علي ناصر، باسل عبدالزهرة عباس*، علي بلبول الديوان

قسم الأحياء الدقيقة، كلية الطب البيطري، جامعة البصرة، العراق

الملخص

بروتيوس ميرابيليس (Proteus mirabilis) هو مُمْرِض انتهازي سالب الجرام، شائع التسبب في التهابات المسالك البولية لدى البشر والحيوانات. هدفت هذه الدراسة إلى عزل وتحديد بروتيوس ميرابيليس من عينات بول البشر والقطط والكلاب، وتقييم وجود جينات ضراوة مُختارة وتنوعها الجيني. جُمعت 150 عينة بول (50 عينة من كل نوع من أنواع العائل) باستخدام تقنيات معقمة. أُجري العزل على أجار HiCrome UTI، وتم تأكيد الهوية باستخدام نظام VITEK-2. استُخدم تفاعل البوليميراز المتسلسل (PCR)للكشف عن أربعة جينات ضراوةureC :، وhpmA، وmrpA، وureR، تلا ذلك التسلسل وتحليل SNP. من بين 150 عينة، تم تحديد 18 عينة على أنها بروتيوس ميرابيليس. كشف تسلسل الحمض النووي عن تباين في أعداد تعدد أشكال النوكليوتيدات المفردة (SNPs) بين الجينات، حيث أظهر جين mrpAأعلى تعدد أشكال، مما يشير إلى تنوع جيني محتمل وآليات تكيف. تُبرز هذه النتائج الإمكانات الحيوانية لبكتيرياP. mirabilis ، وتُؤكد على أهمية التوصيف الجزيئي لفهم ضراوتها وديناميكياتها التطورية في مختلف العوائل.

الكلمات المفتاحية: Proteus mirabilis؛ UreR؛ HpmA؛ MrpA؛ UreC