Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2025, 38 (1)

Molecular Detection of the *hpmA* and *ureA* Genes in Clinical *Proteus Mirabilis* Isolates

Rosa Kareem Shameel[1*] and Marwa Hameed M. Alkhafaji[2]
[1,2]Department of Biology, College of Sciences, University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

Received: 29 May 2023 Accepted: 12 July 2023 Published: 20 January 2025
doi.org/10.30526/38.1.3545

Abstract

Proteus mirabilis is one of the most common Gram-negative bacteria that can cause UTIs. The purpose of this research was to identify virulence genes in *P. mirabilis* strains obtained from the urine of Iraqi patients diagnosed with urinary tract infections. From October 2022 to February 2023, AL-Imam Ali Hospital collected 100 urine specimens from patients exhibiting symptoms and signs of UTI. All specimens were identified based on morphological characteristics on media of culture like pale, non-lactose ferment colonies on MaCconkey agar, swarming on blood agar, and salmonella shigella agar for hydrogen sulfide production, more diagnosed via utilizing molecular detection and the VITEK system, giving positive results for twenty *P. mirabilis* isolates. The (*ureA*, *hpmA*) gene detection results revealed the presence of *ureA* in 18 cases (90%), and *hpmA* in 17 cases (85%). The molecular technique showed that the *ureA* and *hpmA* genes associated with virulence factor hemolysin (hpmA) and urease (ureA) genes are necessary for causing urinary tract infections.

Keywords: *Proteus mirabilis*, virulence, hemolysin, urease, swarming.

1. Introduction

Proteus species are facultative anaerobic, gram-negative, rod-shaped bacteria. It may ferment sugars other than lactose and produce urease. This genus belonging to the Enterobacteriaceae family, and exhibits a positive catalase and nitrase reaction but a negative oxidase reaction, indicating its active movement without spore generation. Certain tests, like phenylalanine deaminase tests and positive urease (1, 2), can be used to identify *Proteus* species. One of the most frequent Gram-negative bacteria that can cause UTIs is *Proteus mirabilis* (3). *P. mirabilis* may cause catheter blockage, stones of the kidney, bacteriuria, acute pyelonephritis, and fever. It is true that *P. mirabilis* strains cause the vast majority of severe UTIs (4, 5). *P. mirabilis* encodes multiple

virulence genes (6, 7). Many different virulence factors contribute to the pathogenicity of *P. mirabilis*. The ability of bacteria for adhesion, colonization, and invading of tissues is promoted by a number of factors, including the existence of lipopolysaccharides, cell invasiveness, environmental iron binding, urease activity, swarming motility, the presence of flagella-based proteins and fimbriae, and hemolysin (8, 9). Important in the pathophysiology of *P.mirabilis*, the urease enzyme catalyses the production of kidney and bladder stones and can also obstruct indwelling urinary catheters (10). The hpmA and hpmB hemolysin proteins are associated with *P. mirabilis* hemolysis. For the protein to become active and cause tissue injury, it must cleave off the N-terminal peptide of hpmA. Previous research suggests that *P. mirabilis* hemolysin has a crucial role in UTI, which leads to *P. mirabilis* potential urovirulence (11). The purpose of this study was to identify virulence genes in *P. mirabilis* obtained from the Iraqi patients39; urine diagnosed with urinary tract infections.

2. Materials and Methods

2.1. Collection of specimens

From October 2022 through February 2023, 100 urine samples were obtained from UTIS patients at AL-Imam Ali Hospital utilizing transport media swabs. Bacterial analysis will be performed on the samples that were sent to the laboratory. This study was approved by the ethical committee of the Biology Department-College of Sciences/University of Baghdad according to the reference number (CSEC/0922/0099). The samples were collected after the approval of the patients.

2.2. Isolation and Identification of *P. mirabilis*

Salmonella shigella (S.S.) agar, MaCcnokey agar, and blood agar were all used to incubate the samples aerobically for 24 hours at 37 degrees Celsius. Gramme stain was used to determine the bacterial reaction to stain, arrangement, and form (12), allowing for identification based on microscopical features. Swarming on blood agar, non-lactose ferment on MaCconkey agar, and hydrogen sulfide generation on S.S agar are all morphological traits that may be observed when growing bacteria in the laboratory. The *Proteus* isolates were identified using a battery of biochemical assays, including the indole test (13) and the urease test (14), before their identities were eventually validated using the Vitek2 system (15).

2.3. Genetic study

2.3.1. Genomic DNA extraction

Isolates of *P. mirabilis* were grown in Mueller-Hinton broth for 24 hours at 37°C, and DNA was extracted using a commercial kit. Bacterial growth DNA was extracted using the Easy Pure genomic DNA kit. The purity and quality of the DNA were tested using a Qubit 4.0 assay.

2.3.2. Quantitation of DNA by Qubit 4.0

The test is sensitive to samples with concentrations between 10 pg/L and 100 ng/L and prefers double-stranded DNA (dsDNA) over RNA. The test generates the three-hour signal at room temperature. The test permits many common contaminants, including salts, free nucleotides, solvents, detergents, and proteins.The Qubit® dsDNA HS Reagent was diluted 1:200 in Qubit® dsDNA HS buffer to create theQubit® working solution. Each standard tube had 190 µL of Qubit®

working solution added to it,and then 10 µL of each standard solution was added to it and vortexed. Each sample tube had 197µL of the Qubit® working solution and 3 µL of sample added to it. After incubating all of the ingredients in a vortex mixer for 3 minutes at room temperature, we tried them. Standards tubes were used in the Qubit device to generate a concentration curve. Sample tubes were introduced sequentially to the dsDNA concentration reader.

2.3.4. Primers

In this study, specific primers were designed to identify virulence genes (*ureA* and *hpmA*) in *P. mirabilis* (Table 1). These genes were amplified using a multiplex PCR technique.

Table 1. Sequences of primer and their sizes utilized to reveal specific genes of virulence factors.

Genes	Sequences of primer (5→3)	Size(bp)
HpmA	R-CTACTCGCTACTAATGTGATG	240
UreA	F-CTCGTATTGATAGTAGAGGGA R-AGGTGAGTGAATTGAAACC F-GTTGCAGAAAGACGTTTAG	400

R: Reverse primer, F: Forward primer

The genes were amplified depend on program of PCR in **Table 2**.

Table 2. Program of PCR for amplification of genes.

Cycle No.	Stage	Temperature	Time
1X	Initial Denaturation	94 ºC	5mint
	Denaturation	94ºC	30sec
30X	Annealing	46.5ºC	45sec
	Extension	72ºC	45sec
1X	Final Extension	72ºC	7mints

2.3.5. Mixture reaction

The PCR amplification mixture contains Master mix (12.5 microliters), forward primer1 (1 microliter), reverse primer1 (1 microliter), forward primer2 (1 microliter), reverse primer2 (1 microliter), nuclease-free water (3.5 microliters), and DNA template (5 microliters).

The conditions of PCR were as follows: initial denaturation at 94°C for five minutes, followed by denaturation at 94°C for thirty seconds, annealing at 46.5°C for forty-five seconds, extension at 72°C for forty-five seconds, and final extension at 72°C for seven minutes.

2.3.6. Agarose gel electrophoresis

To make the agarose gel, we first dissolved 1.2 grams of agarose powder in 60 milliliters of 1xTBE buffer by heating the solution in the microwave until all of the gel particles dissolved. The agarose solution was cooled to 70° C, and then 4 l of RedSafeTM was added and well incorporated. Gel was put into the gel tray and allowed to settle at room temperature (20-25 °C)for 30 minutes before the combs were removed. The jar was then placed in the

electrophoresis tank. The first well of the agarose electrophoresis gel included 8 µL of the DNA ladder, while the subsequent wells had 8 µL of each PCR product. Next, we put the lid on the electrophoresis tank and set the electric current (80 volts for 80 minutes). The redsafe-stained bands in the gel were visualized with a gel documentation system.

3. Results

3.1. Bacterial isolation

Morphological aspects of culture traits and biochemical parameters are used extensively in isolating and identifying *P. mirabilis*. One hundred distinct samples of urine were grown on several different mediums. Plates of S. S. agar and MacConkey *ureA* base agar, as well as blood, were among them. *P. mirabilis* colonies on blood agar, for example, may be recognized by the clustering phenomena in which they grow in close proximity to one another, as well as by their distinctive fishy odour. Positive results are shown in **Figures** (**1-4**) for 20% of the isolates of *P. mirabilis* that grow in continuous waves, forming concentric thin film layers (swarms). Colonies on Macckongy agar are round, pale, and do not appear to form colonies (15).

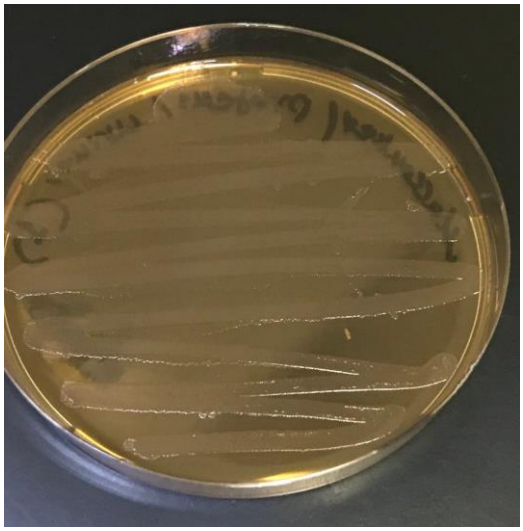

Figure 1. *Proteus mirabilis* on Macckongy agar after18- 24h of incubation at 37°C, the colonies appear are smooth and pale colonies.

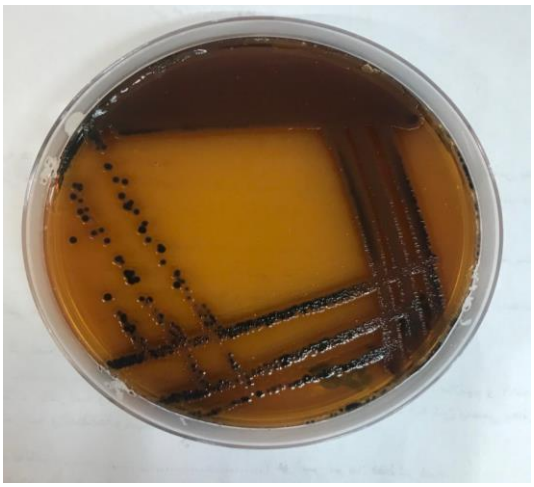

Figure 2. *Proteus mirabilis* on *Salmonella Shigella* agar after 24h of incubation at 37°C, the colonies appear pale with black center (hydrogen sulfide production).

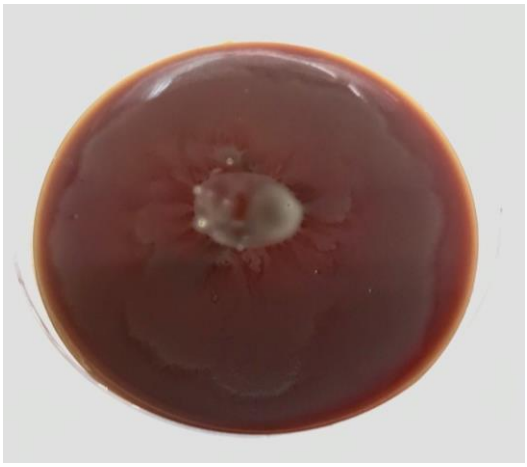

Figure 3. *Proteus mirabilis* on blood agar after 24h of incubation at 37°C (swarms and alpha hemolysis).

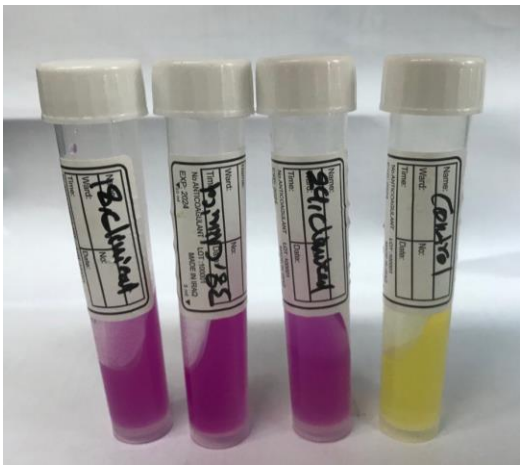

Figure 4. *Proteus* inoculated into Christensen medium which give positive results pink color after 24h of incubation at 37°C**.**

3.2. Identification by using VITEK System

In addition, the Vitek2 system's results for identifying *P. mirabilis* showed that all isolates were indeed *P. mirabilis*, with a percentage of identification ranging from (95 to 99%).

3.3. Results of the multiplex PCR

It works the same way with *ureA* and *hpmA*-specific primers, morphological methods, and the Vitek2 system to identify all 20 *P. mirabilis* isolates. The picture in **Figure 5** shows how a single band with a known molecular weight (240 bp for *ureA* and 400 bp for *hpmA*) can be used as a sign to find genes. There were a total of 17 (85%) and 18 (90%) positive isolates for the presence of *hpmA* and *ureA*, respectively. Those are evidenced by Table 3.

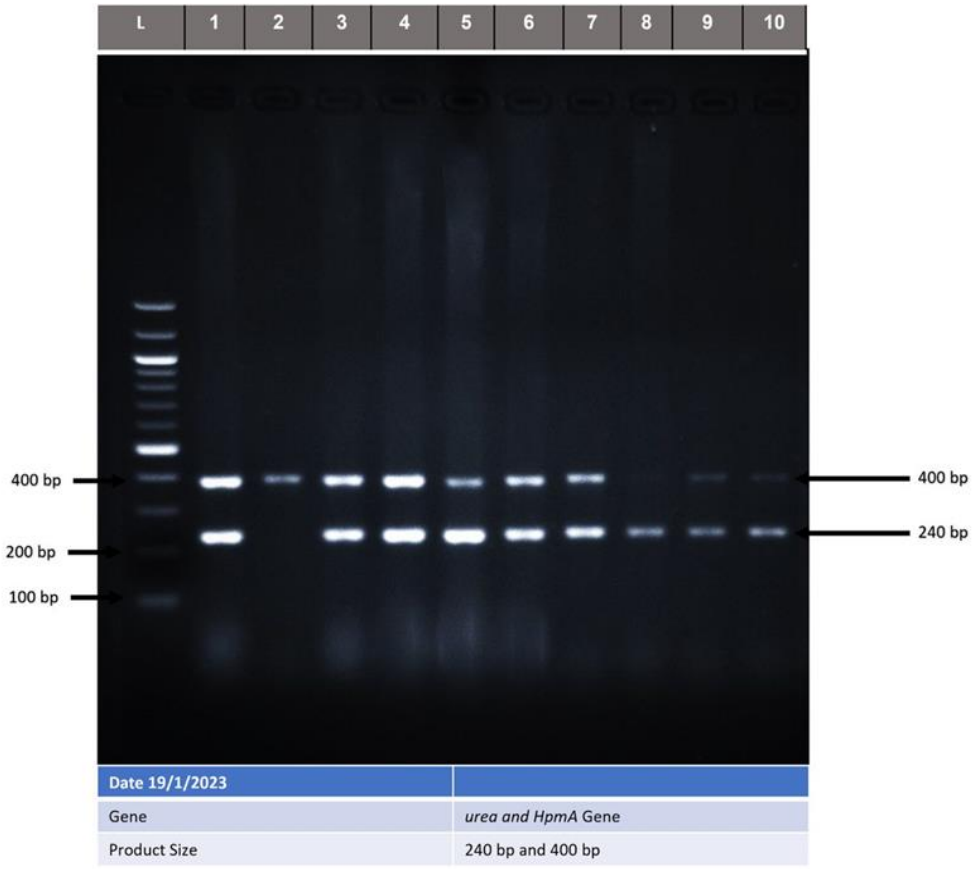

Figure 5. Multiplex PCR for *ureA* and *hpmA* (2% agarose gel, TBE buffer, 80 mints). *UreA* gene (size 240 bp) and *hpmA* gene (400) extraction 85 volts two percent of agarose gel electrophoresis for 80 mints. L: Ladder with 1000bp**.**

Table3. Prevalence of virulence genes (*hpmA*, *ureA*) of *P. mirabilis.*

Genes	Number of isolates	percentage %
HpmA	17	85%
UreA	18	90%

4. Discussion

Twenty isolates were found by testing their characteristics on various mediums (blood agar, S. S agar, and MacConkey agar). *P. mirabilis* was confirmed by colonisation on blood agar, a fishy odor, and the appearance of smooth, pale colonies on MacConkey agar before being diagnosed with the VITEK2 system and molecular detection.

All of them demonstrated phenotypical signs of extracellular urease positivity. These results are consistent with those observed by (17–24), who also found that all *P. mirabilis* isolates tested strongly produced urease. One of the most crucial aspects of *P. mirabilis* pathophysiology is urease. When grown in a lab (on basic ureA agar), urease changes ureA into carbon dioxide and alkaline ammonia. This raises the pH and turns the phenol red indicator pink (25). However, *in vivo* (inside a human's body), this enzyme catalyzes the production of bladder and kidney stones, or encrustation and obstruction of the indwelling urinary system (26, 27).

Different types of biochemical and morphological tests back up the finding that *ureA* levels are lower in 18 (90%) of clinical isolates compared to 28 (100%) and 29 (10%). Urease, the primary enzyme in both kidney and bladder stone formation, is produced by a gene that is required in *P. mirabilis*. Specifically, this *ureA* gene is required (30).

Additionally, 17 out of 28 people tested positive for HpmA, an increase of 85% from the previous result of 5 (22.72%). Prior research indicated that the frequency of the *hpmA* gene was 30% (31), which is quite close to the values described here. The *hpmA* gene is one virulence factor that contributes to UTI development. The fact that this gene also contributes to infections in other parts of the urinary system makes it noteworthy.

The *P. mirabilis* bacterium secretes a hemolysin that is toxic to kidney tissue. *Two genes, hpmA and hpmB,* regulate HpmA, a larger, calcium-independent protein with an N-terminal peptide (166 kDa). Calcium-independent hemolysin leaking through the pores may activate HpmA (140 kDa) (32). The invasiveness of *P. mirabilis* strains coincides with increased HpmA production, which undergoes coordinated control throughout cell development into colony forms and infection (33).

5. Conclusion

According to the results of this study, *P. mirabilis* isolates from urinary tract infections have the ability to possess virulence factors (phenotypic and genotypic), such as urease and hemolysin.

Acknowledgment

We would like to extend our gratitude to the University of Baghdad and .AL-Imam Ali Hospital for supporting this research.

Conflict of interest

The authors declare that they have no conflicts of interest.

Funding

self-funded.

Ethical Clearance

This work was approved by the ethical committee of the Department of Biology / College of science / Baghdad University according to the Ref number (CSEC/0922/0099). The specimens were collected with the approval of the patients.

References

1. Mordi RM. , Momoh MI. Incidence of *Proteus* species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. Afr. J. Biotechnol. 2009; 8(5): 725-730 https://doi.org/10.5897/AJB2009.000-9123
2. Hassoon SJ. , Mohamed WM. , Mohsen A. Isolation and Identification of *Proteus* bacteria from mouth and nose of human and animals with studying the Sensitivity to ethanol: aqueous extracts of pomegranate peel and Lantana cammara leaves. Journal of Education and Scientific Studies. 2021; 3(17).
3. Salumi ZN., Abood ZH. Phenotypic Diagnosis of Efflux Pump of Escherichia coli Isolated from Urinary Tract Infections. Iraqi Journal of Biotechnology. 2022; 21(2):21-31.
4. Schaffer JN., Pearson MM .*Proteus mirabilis* and urinary tract infections. Microbiology Spectrum. 2015; 3.
5. Al-obaidi SA. , Al-Hashimy AB. Molecular Screening for luxs and pm1Virulence Genes of *Proteus mirabilis* Isolated from Iraqi Urinary Tract Infection Patients. Iraqi Journal of Biotechnology. 2022; 21(2):499-504.
6. Abbas KF. , Al Khafaji JK. , Al-Shukri MS . Molecular detection of some virulence genes in *Proteus mirabilis* isolated from hillaprovince. International Journal of Research Studies in Biosciences. 2015; 3: 85–89.
7. Morgenstein RM. , Szostek B. , Rather PN .Regulation of gene expression during swarmer cell differentiation in *Proteus mirabilis*. FEMS Microbiology Reviews. 2010; 5(34): 753–763.
8. Rózalski A., Sidorczyk Z. , Kotełko K. Potential virulence factors of Proteus bacilli. Microbiol Mol Biol Rev. 1997; 61:65–89. https://doi.org/10.1128/mmbr.61.1.65-89.1997
9. Legnani-Fajardo C., Zunino P., Piccini C. , Allen A. , Maskell D. Defined mutants of *Proteus mirabilis* lacking flagella cause ascending urinary tract infection in mice. Microb Pathog 1996; 21:395–405.
10. Armbruster CD. , Mobley HLT. , Pearson MM .Pathogenesis of *Proteus mirabilis* infection. EcoSal Plus. 2018; 8(1) . https://doi.org/10.1128/ecosalplus.esp-0009-2017
11. Cestari SE. , Ludovico MS. , Martins FH.S. , da Rocha PD. ,Elias WP. , Pelayo JS. Molecular detection of *HpmA* and HlyA hemolysin of uropathogenic *Proteus mirabilis*. Current Microbiology. 2013; 67(8): 703–707. https://doi.org/10.1007/s00284-013-0423-5
12. Al-Ani DA. ,Abdullah AH. Molecular Detection of locally Bacillus cereus isolated from Bovine milk. Current Research in Microbiology and Biotechnology. 2017;*5*(5): 1233-1238.
13. Hara CM. , Brenner FW. , Miller JM. Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clinical Microbiol. 2000;13(4):534-546. https://doi.org/10.1128/cmr.13.4.534-546.2000
14. Ismail HY., Ijah UJ., Riskuwa M.L., Allamin II. Biodegradation of spent engine oil by bacteria isolated from the rhizosphere of legumes grown in contaminated soil. International Journal of Environment. 2014;3(2):63-75.
15. Al-obaidi SA. ,Al-Hashimy AB. Molecular Detection of (Urec, Mrpa, *HpmA*) Genes in *Proteus mirabilis* Bacteria Isolated From Patients .Hospital Medicine. 2023;90(1):716-720.

16. Husain MF., Kandala NJ. Isolation and Identification of Salmonella spp. Using Molecular and Phenotypic Methodes. Iraqi Journal of Biotechnology. 2022;21(2):550-560.
17. Fox-Moon SM., Shirtliff ME. Urinary tract infections caused by *Proteus mirabilis*. In Molecular Medical Microbiology. 2015: 1389-1400,Academic Press.
18. Jabur MH., AL-Saedi EA., Trad JK . Isolation of *Proteus mirabilis* and Proteus vulgaris from different clinical sources and study of some virulence factors. J. of Bab. Univ. pure and applied sciences. 2013;21(1):43-48.
19. Al-Duliami AA., Nauman NG. , Hasan ARS. , Al-Azawi ZH. Virulence factors of *Proteus mirabilis* isolated from patients otitis media in Baquba and it's peripheries. Diyala journal of Medicine. 2011; 1(1):.69-75.
20. Filipiak A. , Chrapek M., Literacka E., Wawszczak M., Głuszek S., Majchrzak M., Adamus-Białek W. Pathogenic factors correlate with antimicrobial resistance among clinical *Proteus mirabilis* strains. Frontiers in microbiology. 2020; 11, 579389.
21. Hezam AM. Molecular characterization of *Proteus mirabilis* virulence factors isolated from patients with otitis media in Diwaniyah, Iraq. Al-Qadisiyah Journal of Pure Science. 2021;26(5): 11-22..
22. Barbour EK., Hajj ZG., Hamadeh S., Shaib HA., Farran MT., Araj G.,Faroon O., Barbour KE., Jirjis F., Azhar E. , Kumosani T. Comparison of phenotypic and virulence genes characteristics in human and chicken isolates of *Proteus mirabilis*. Pathogens and global health. 2012;106(6): 352-357.
23. ABID ASAAJ. , ABBAS FM. Molecular assessments of *Proteus mirabilis* virulence factors isolated from urinary tract infection patients. International Journal of Pharmaceutical Research. 2018; 10(4).
24. Ali HH. , Yousif MG. Detection of some virulence factors genes of *Proteus mirablis* that isolated from urinary tract infection. IJAR. 2015; *3*(1):156-163.
25. AL-Oqaili NA., AL-Shebli MK., Almousawi AN. Antimicrobial susceptibility and molecular characterization for some virulence factors of *Proteus mirabilis* isolated from patients in Al-Qadisiyah Province/Iraq. Al-Qadisiyah Journal of Veterinary Medicine Sciences. 2017, Dec 30; 16(2):1-7.
26. Johnson DI. , Johnson DI. *Proteus* spp. Bacterial Pathogens and Their Virulence Factors 2018:317-323.
27. Khalaf TT., AL-Hashimy AB. Molecular Identification of *Klebsiella pneumonia* Isolated from UTI Patientsin Al-Anbar Governorate and Study Its Antibiotic Resistance and determination of Antimicrobial Activity of Flaxseed Oil. Iraqi Journal of Biotechnology. 2022;21(2):612-622.
28. Al-obaidi SA. , Al-Hashimy AB. Molecular Detection of (Urec, Mrpa, *HpmA*) Genes in *Proteus mirabilis* Bacteria Isolated From Patients with Urinary Tract Infection. The Egyptian Journal of Hospital Medicine. 2023;90(1): 716-720.
29. Al-mayhi FS. Phenotypic and Molecular detection of Virulence factors in *Proteus mirabilis* isolated from different clinical sources. Basrah Journal of Veteirnary Research.2017;16(1): 369-388
30. Sanches MS., Silva LC., Silva CRD. , Montini VH., Oliva BHDD.,Guidone GH.M.;Nogueira, MCL.,Menck-Costa MF., Kobayashi RKT.,Vespero EC. , Rocha SPD. Prevalence of Antimicrobial Resistance and Clonal Relationship in ESBL/AmpC-Producing *Proteus mirabilis* Isolated from Meat Products and Community-Acquired Urinary Tract Infection (UTI-CA) in Southern Brazil. Antibiotics. 2023; 12(2): 370.
31. Lazm A., Jebur M, Alomashi G. Sequencing of *HpmA* Gene in *Proteus mirabilis* of UTIs among rheumatoid arthritis patients. J Pharm Sci Res. 2018;10(2):265- 71.
32. Al-Hamdani H., Al-Hashimy A. Molecular detection of UREC, *HPMA*, RSBA AND MRPA genes of *Proteus mirabilis* urinary tract infection in patient with rheumatoid arthritis. Iraqi Journal of Agricultural Science. 2020; 51:245-51.

33. Abed MK., Shareef HK. Isolation and Molecular Identification of *Proteus mirabilis* isolated from hospitals in the capital Baghdad. Indian Journal of Forensic Medicine & Toxicology. 2021;15(1):.2216-2223.