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Original Article

Molecular Study of Some Virulence Genes in *Proteus Mirabilis* Isolated from Urinary Tract Infection Patients in Sulaymaniyah, Kurdistan Region/Iraq

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ABSTRACT

Urinary tract infections (UTIs) are a severe public health issue that are caused by a variety of pathogens, including *Proteus mirabilis*, it is the main bacterium which causes complicated (UTIs), mainly catheter-associated UTIs (CAUTIs). This study aimed to identify genes that regulate pathogenic virulence factors such as hemolysin, urease, protease enzyme activity, biofilm formation and fimbriae of *P. mirabilis* isolated from clinical samples in Sulaymaniyah hospitals. (117) urine samples collected from five hospitals were studied. Gender characteristics revealed that the majority of samples were from female patients (70, 59.8%); meanwhile, 47 (40.1%) were from male patients. No statistically significant correlation was found between gender and the incidence of urinary tract infections (UTIs) (Fisher's Exact Test, p = 0.56). Although, Females had a marginally greater prevalence of UTI (92.9%, 65/70) than males (89.4%, 42/47), however this difference lacked statistical significance. Results showed that *E. coli* was present in 17 (14.5%), *S. hemolyticus* in 11 (9.4%) isolates, and *P. mirabilis* in 10 (8.5%). Molecular diagnosis was confirmed using PCR targeting (16S rRNA) gene, followed by DNA sequencing, and then registered in Gene Bank-NCBI; also, accession numbers were gained. Specific primers were chosen for genes (UreC, MrpA, ZapA, HpmA, LuxS). Results demonstrated that all ten isolates possessed five mentioned genes, with the exception of HpmA, which was lacking in two isolates. All Proteus mirabilis isolates demonstrated multidrug resistance (MDR), indicating a potential correlation between MDR and the expression of virulence factors, such as hemolysin synthesis and biofilm formation, among the isolates. Sequencing for the virulence genes was performed and compared to NCBI database registered sequences.

Keywords: (UTI), Proteus mirabilis, (MDR), virulence factors, Bacteriuria, (CAUTI).

1 Introduction

Bacterial proliferation in the normally sterile urinary tract (kidney and bladder) is referred to as a urinary tract infection (UTI). The condition can range from asymptomatic bacteriuria to a severe kidney infection that may result in sepsis [1]. The bladder urothelium is crucial in the host's innate immune response to (UTI). Women are particularly susceptible to these infections due to several factors, including the influence of estrogen on urothelial defense mechanisms and the shorter distance from the urethral opening to the bladder. These factors raise the possibility that a potential uropathogen may get to the bladder, proliferate in the urine, and invade the bladder wall or progress to the kidneys, resulting in a (UTI) [2]. *Proteus mirabilis* is a Gram-negative, rod-shaped facultative anaerobe bacterium. It belongs to the Gammaproteobacteria class and is part of the Enterobacteriales order and Enterobacteriaceae family. This pathogen is known for its unique urease production, swarming behavior, and fast multicellular activity, it causes (UTIs) and catheter associated (UTIs) [3]. Numerous virulence factors have been found and characterized in *P. mirabilis* which involve a powerful urease regulated by (*Ure*) gene that facilitates the conversion of urea to ammonia resulting in production of urinary stones [4]. The extracellular metalloprotease *(ZapA)*, which is also known as an (IgA) protease, is one of the numerous mechanisms that this bacterium employs to evade host defenses and is expressed in numerous strains of *P. mirabilis*. *(ZapA)* expression is coordinated with swarming cell development and swarming behavior. The hemolytic activity generated by this bacterium is linked to hemolysin (*HpmA*). It is cell-associated, calcium-independent and forms pores which is encoded by two genes, (*HpmA*) and (*HpmB)* producing (166 kDa) and proteins, respectively [3]. There is limited published research which detects virulence genes in *P. mirabilis* isolates from Sulaymaniyah Province, consequently this study addresses these gaps by performing initial PCR-based screening of (*UreC, MrpA, HpmA,*

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ZapA, and LuxS) genes establishing local prevalence standards for virulence factors supplying data for guidance specific therapeutic procedures.

2 Materials and methods

2. 1 Study design and setting

This research was a descriptive molecular analysis conducted to investigate prevalence of virulence factors in *P. mirabilis* isolates from (UTIs) by PCR based methods. The study was carried out at five tertiary hospitals (Shar, Hiwa, Sulaymaniyah Teaching Hospital, Baxshin & Harem) in Sulaymaniyah city extended from September 2024 to January 2025 as duration period.

2. 2 Ethical considerations

The study received approval from the Ethical Committee in College of Science at University of Charmo and Sulaimani General Directorate of Health on 26 September 2024 (reference No:6). The urine samples were obtained from individuals diagnosed with urinary tract infections (UTIs) at hospitals of Sulaymaniyah province. Only broad demographic information (e.g. age, sex,) was obtained and all data was treated as strictly confidential. No individually identifiable information was seen. Samples utilized in this investigation were initially obtained for regular diagnostic procedures and their research use was undertaken in conformity with institutional protocols and ethical standards.

2. 3 Sample collection

A total of (117) urine samples were collected from patients with suspected (UTIs) in which cultured on (Blood agar, MacConkey agar) with incubation for 24 hours at 37 ℃ [5].

2. 4 Statistical analysis

Statistical analysis was conducted with GraphPad Prism. The correlation between gender and the incidence of urinary tract infections (UTIs) was assessed utilizing Fisher's Exact Test.

2. 5 Bacterial identification

Initial selection of *P. mirabilis* isolates was based on distinct colony characteristics as swarming growth on blood agar and non-lactose fermenting colonies on MacConkey agar [5]. Further identification was performed to confirm *P. mirabilis* isolates using biochemical tests, including urease test [6].

2. 6 Automated identification systems

For more accuracy, VITEK2 system (Bio Merieux/France) and BD Phoenix™ system (Canada) were used to confirm bacterial identification. Out of (117) urine samples, 10 were identified as *P. mirabilis* and selected for further molecular analysis.

2. 7 DNA Extraction and quality assessment

Bacterial genome was extracted from overnight cultures of ten *P. mirabilis* isolates using the Bacterial Genomic DNA Extraction Kit (Add Bio™/Korea). The DNA concentration and purity were measured using a Nanodrop spectrophotometer (Thermo Scientific™/USA). To verify the integrity of the extracted DNA, gel electrophoresis was performed (1.5% agarose gel at 75 volts for one hour) stained with Ethidium Bromide, producing clear DNA bands for all 10 isolates.

2. 8 Molecular confirmation and identification of isolates

PCR amplification was performed using primers targeting the *16S rRNA* gene. The PCR products were sequenced to confirm the identity of the isolates (Tables 1 & 2). Selected sequences were compared with reference sequences from NCBI GenBank. The isolates exhibited significant similarity to the NCBI database and were identified as *P. mirabilis* based on sequence similarity. Once the sequences were compared, they were all uploaded to the NCBI GenBank and accession numbers were gained.

Table 1: Primer set used for 16S rRNA gene amplification.

Primer sequences (5'- 3')	Amplicon length (bp)	Ref.
F: 5'-CCTGGACAAAGACTGACGCT-3' R: 5'-CGCTTCTCTTTGTATGCGCC-3'	523	[7]

Table 2: Thermal cycle's program of PCR conditions for 16S rRNA amplification.

Step	Temperature (°C)	Time	Number of Cycles
Initial Denaturation	95	10 min.	1
Denaturation	95	30 sec.	30
Annealing	60	30 sec.	30
Extension	72	17 sec.	30
Final Extension	72	5 min.	1

2. 9 Phylogenetic Evaluation:

Examining the evolutionary links of selected bacterial strains, a phylogenetic tree was constructed using MEGA (V 11.0) by neighbor-joining test. The 16S rRNA sequences were manually examined for poorly aligned regions to ensure high-quality data for tree construction, and the poorly aligned end regions were trimmed.

2. 10 Detection of Virulence Factor Genes

Subsequent PCR assays were carried out using specific primers to detect five key virulence genes associated with *P. mirabilis* including (*UreC, MrpA, ZapA, HpmA, LuxS*) (Tables 3 & 4). PCR products were analyzed by gel electrophoresis (1.5% agarose, 75 volts, 1 hour) stained with Ethidium Bromide, the size of all amplicons was determined by comparing them to a DNA ladder 1000 bp (Genesand Biotech Co. ltd/China).

Table 3: Primer sequences and product sizes used to detect five virulence genes.

Gene	Primer sequences (5'- 3')	Amplicon length (bp)	Ref.
HpmA	F:5'- GTTGAGGGGCGTTATCAAGAGTC-3' R:5'-GATAACTGTTTTGCCCTTTTGTGC-3'	709	[8]
UreC	F:5'-CAAGCCCAAGAAGGTCTCGT-3' R:5'-CAAGATGCTCGTCCACGGTA-3'	517	[9]
ZapA	F:5'-ACCGCAGGAAAACATATAGCCC-3' R:5'-GCGACTATCTTCCGCATAATCA-3'	540	[10]
MrpA	F:5'-TTCTTACTGATAAGACATTG-3' R:5'-ATTTCAGGAAACAAAAGATG-3'	565	[11]
LuxS	F:5'ACGTATGTCTGCACCTGCG-3' R:5'CCATAGCTGCCTTCCATGCA-3'	290	[12]

Table 4: Thermo cycle's program of PCR conditions (30 cycles) for five virulence gene amplifications.

Gene	Amplicon length (bp)	PCR conditions	Ref.
HpmA	709	Initial Denaturation: 95°C for 10 min. Denaturation: 95°C for 30 sec. Annealing: 60°C for 30 sec. Extension: 72°C for 29 sec. Final Extension: 72°C for 5 min.	[8]
UreC	517		[9]
ZapA	540		[10]
MrpA	565		[11]
LuxS	290		[12]

2. 11 DNA Sequencing and Analysis

The amplified PCR products were sent for DNA sequencing using the SeqStudio/USA Genetic Analyzer. Obtained sequences were compared with reference gene sequences available in NCBI databases to verify the presence and identification of the virulence genes.

3 Results

3. 1 sample collection

A total of (117) urine samples were obtained from patients suspected with urinary tract infections in five hospitals in Sulaymaniyah city. The majority of samples were from females in which formed 70 (59.8%) of samples, in contrast 47 (40.1%) of samples were from males. Results showed that *E. coli* was present in 17 (14.5%), *S. hemolyticus* in 11 (9.4%) isolates, and *P. mirabilis* in 10 (8.5%).

3. 2 Bacterial isolation and identification

Initial culturing and morphological analysis revealed swarming growth on Blood agar and non-lactose fermenting colonies on MacConkey agar by typical *Proteus* spp. characteristics. Biochemical assays, including urease test (figure 1&2) verified 10 of the samples as *P. mirabilis* which were (2) and (8) from males and females, respectively; demonstrating a larger proportion of females among UTI patients. Patient's age ranged from (1.8 to 82) years, representing pediatric, adult, and senior age groups. All patients had received a clinical diagnosis of UTI. Regarding catheterization status, four patients were catheterized, whereas six were not. The existence of catheterization emphasizes its function as a possible risk factor for UTI (Table 5).

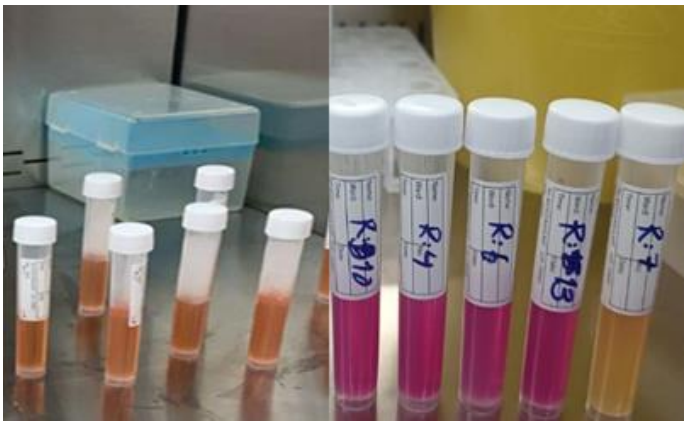

Figure 1: The tested *Proteus mirabilis* isolates showed strong urease positivity, which is considered an important virulence factor associated with urinary tract infections, Positive urease activity was observed by the development of a pink color in the urea medium, indicating the hydrolysis of urea into ammonia and carbon dioxide by the enzyme urease, yellow color demonstrated negative urease.

Table 5: Gender, age and catheterization status of *P. mirabilis* isolates.

Sample NO.	Gender	Age (years)	Catheterization
1	Male	59	Yes
2	Female	67	No
3	Female	2	No
4	Male	29	No
5	Female	82	Yes
6	Female	3	No
7	Female	1.8	Yes
8	Female	60	Yes
9	Female	50	No
10	Female	30	No

3. 3 Statistical Analysis of Gender and UTI Incidence

In Fisher's Exact Test, the p-value was 0.56, which indicates that there was no statistically significant association between gender and the occurrence of urinary tract infections (UTIs). Despite the fact that females had a slightly greater prevalence of urinary tract infections (92.9%, 65/70) compared to males (89.4%, 42/47), this difference did not reach statistical significance statistically.

3. 4 Antimicrobial Susceptibility Profile

The antimicrobial-susceptibility profile of the clinical isolates was assessed against (18) antibiotics using the BD Phoenix™ system (Figure 2). Amikacin showed high efficacy; (9/10; 90%) of isolates were sensitive and (1/10; 10%) were resistant. Gentamicin showed moderate activity, in which (5/10; 50%) of isolates half and half were sensitive or either resistant. Overall, (10/10; 100%) of isolates showed high susceptibility to carbapenems (ertapenem and meropenem). ceftolozane-tazobactam showed high activity; (9/10; 90%) were sensitive, and (1/10; 10%) of isolate showed intermediate result. Ampicillin showed low activity in which (4/10; 40%) of isolates were sensitive and (6/10; 60%) were resistant. Amoxicillin-clavulanate exhibited complete susceptibility showing (10/10; 100%) for each antibiotic. On the other hand, piperacillin-tazobactam exhibited total sensitivity with (10/10; 100%) of isolates being sensitive. Colistin exhibited total resistance (10/10; 100%) of isolates. Trimethoprim–sulfamethoxazole, exhibited limited susceptibility in which (3/10; 30%) of isolates were sensitive and (7/10;70%) were resistant. Nitrofurantoin exhibited inadequate efficacy in which (10/10; 100%) of isolates were resistant. Fluoroquinolones exhibited significant resistance: ciprofloxacin and levofloxacin each demonstrated (3/10; 30%) sensitive isolates and (7/10; 70%) resistant isolates. Finally, Tetracycline exhibited totally resistance against (10/10; 100%) of isolates.

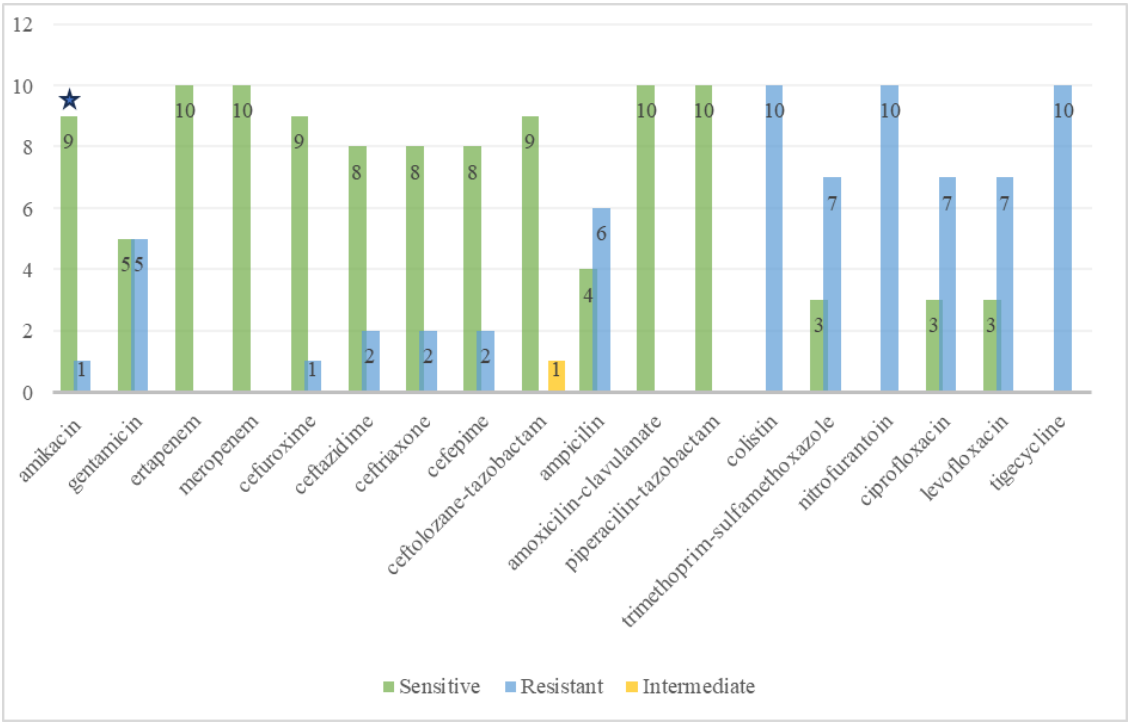

Figure 2: Antibiotic sensitivity test of ten *P. mirabilis* isolates.

3. 5 Multidrug Resistance (MDR) Profiles of Proteus mirabilis Isolates

Among the investigated P. mirabilis isolates, numerous strains demonstrated multidrug resistance (MDR), exhibiting resistance to various antimicrobial classes, including aminoglycosides, β-lactam/β-lactamase inhibitor combinations, polymyxins, folate pathway inhibitors, nitrofurans, fluoroquinolones, tetracyclines, carbapenems, and cephalosporins.

Table 6: Multidrug resistance (MDR) patterns among *Proteus mirabilis* isolates according to antimicrobial classes.

Isolate ID	aminoglycosides	B-lactamase inhibitor	polymyxin	folate pathway inhibitor	nitrofuran	fluoroquinolone	Tetracycline	carbapenems	cephalosporins	MDR
R1	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*YES*
R2	*S*	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*YES*
R3	*S*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*S*	*YES*
R4	*S*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*S*	*YES*
R5	*S*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*S*	*YES*
R6	*S*	*S*	*R*	*S*	*R*	*R*	*R*	*S*	*S*	*YES*
R7	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*S*	*YES*
R8	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*R*	*YES*
R9	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*S*	*R*	*YES*
R10	*R*	*R*	*R*	*S*	*R*	*R*	*R*	*S*	*S*	*YES*

3. 6 DNA Quality Assessment

Quality analysis by nanodrop spectrophotometer showed that DNA concentrations ranged from (60.0 to 180.4 ng/µL) indicating successful extraction from all samples. Variations in genomic yield may be due to differences in sample biomass or extraction efficiency; however, all concentrations were sufficient for downstream molecular applications. The (A260/A280) ratios ranged from (1.88 to 1.93), which is consider within the acceptable range for DNA purity degree, indicating minimal protein contamination. Overall, the obtained DNA quality values confirm the effectiveness of the extraction method and supports its use for subsequent molecular studies (Table 7).

Table 7: DNA quality analysis.

Sample NO.	**Concentration\ ng/µL**	**Purity (A260/A280)**
R1	71.3	1.90
R2	67.0	1.89
R3	76.9	1.89
R4	60.3	1.93
R5	146	1.89
R6	180.4	1.88
R7	142	1.89
R8	60	1.89
R9	123	1.9
R10	62.7	1.89

3. 7 Molecular Confirmation

Genomic DNA extracted from all isolates exhibited adequate concentration and purity for subsequent analysis. Integrity assessments utilizing 1.5% agarose gel electrophoresis yielded distinct bands for each sample. PCR targeting the (16S rRNA) gene yielded single amplicons from all isolates, so confirming molecular identification as P. mirabilis (Figure 3). The sequence data for these amplicons were submitted to NCBI GenBank, and each isolate received an official accession number (Table 8).

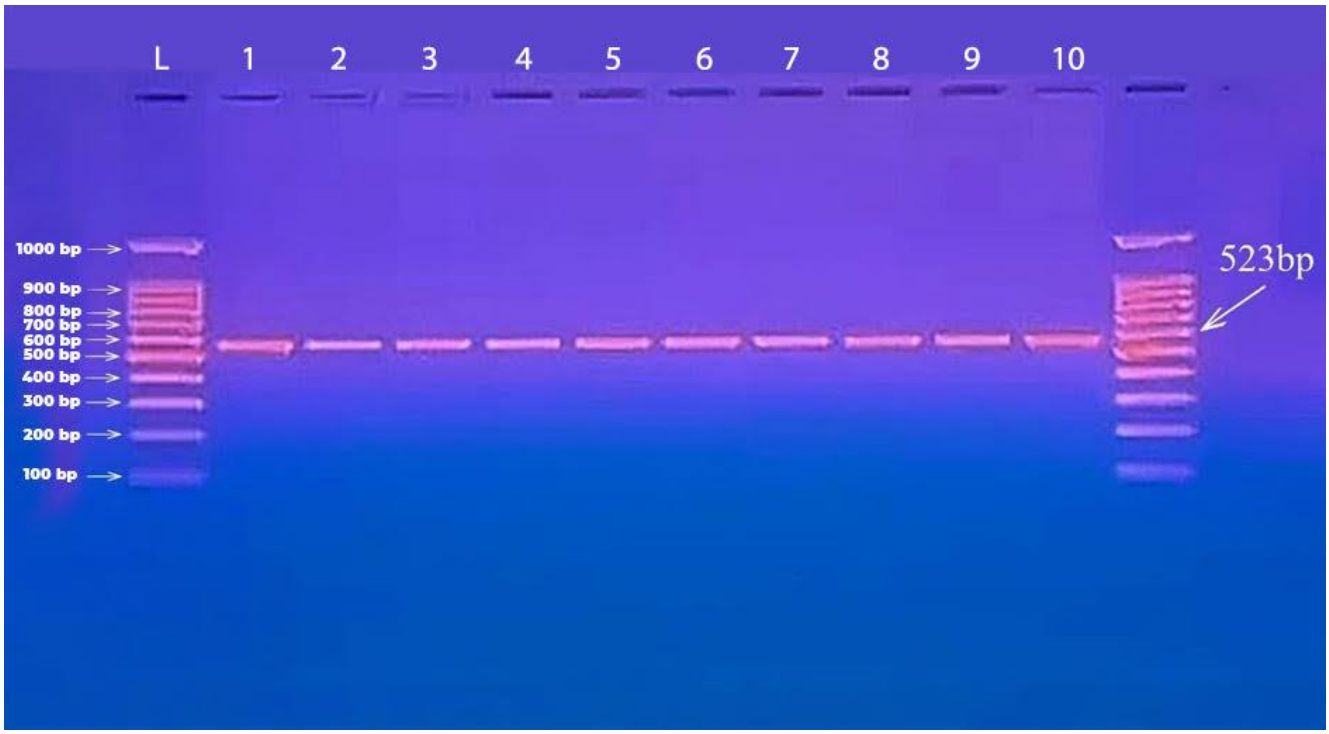

Figure 3: Agarose gel electrophoresis of (16S rRNA) gene of approximately (523 bp), Lanes: L, 100 bp DNA ladder; 1 to 10 are positive sample numbers.

Table 8: Accession numbers of 16S rRNA sequences representing 10 *P. mirabilis* isolates.

Isolate ID	Submission ID	Accession Number
R1	SULYRH1	PX474636
R2	SULYRH2	PX474637
R3	SULYRH3	PX474638
R4	SULYRH4	PX474639
R5	SULYRH5	PX474640
R6	SULYRH6	PX474641
R7	SULYRH7	PX474642
R8	SULYRH8	PX474643
R9	SULYRH9	PX474644
R10	SULYRH10	PX474645

3. 8 Phylogenetic evaluation of clinical isolates

The trimmed phylogenetic tree illustrates the evolutionary relationships among P. mirabilis isolates from Sulaymaniyah (Iraq) in comparison with representative strains from other geographic regions. The analysis showed that most Sulaymaniyah isolates form several closely related subclusters with very short branch lengths, indicating- minimal nucleotide divergence and suggesting recent clonal expansion or limited diversification within this local population (figure 4).

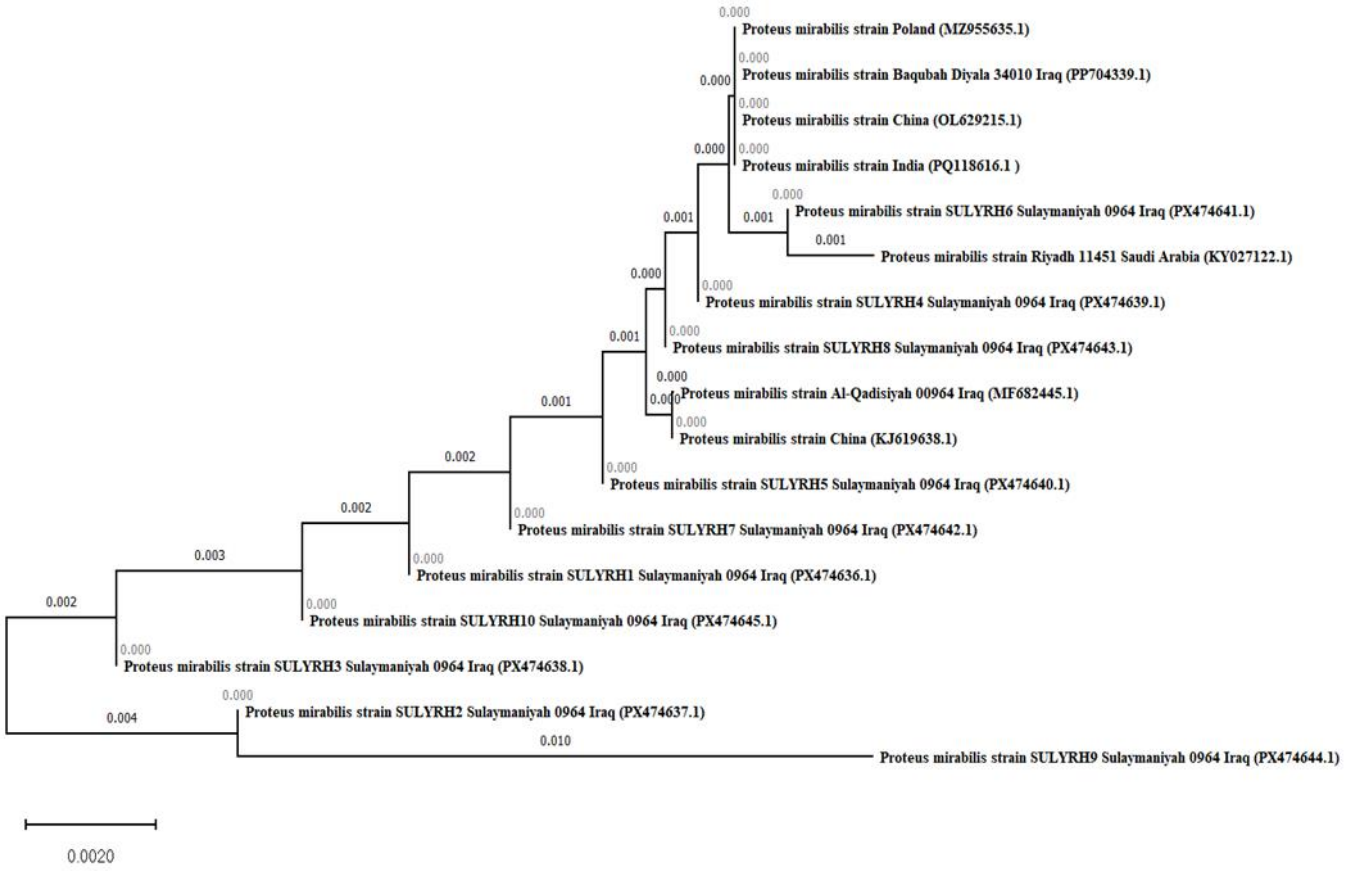

Figure 4: Phylogenetic tree analysis of *P. mirabilis* isolates based on (16S rRNA) gene sequences.

3. 9 Detection of virulence factor genes

Further PCR assays revealed that (100%) of Proteus mirabilis isolates carried screened virulence genes including (UreC, ZapA, MrpA, and LuxS), except (HpmA) gene which was present in eight isolates (80%) as shown by expected product bands in gel electrophoresis for each gene tested. The data indicates that all clinical isolates from this study exhibited essential characteristics associated with, urease activity, protease activity, fimbriae formation, and biofilm formation (Figures 5, 6, 7 and 8). On the other hand, most of isolates had hemolysin characteristics. These molecular results indicate a high virulence potential among P. mirabilis strains in Sulaymaniyah hospitals during the study period.

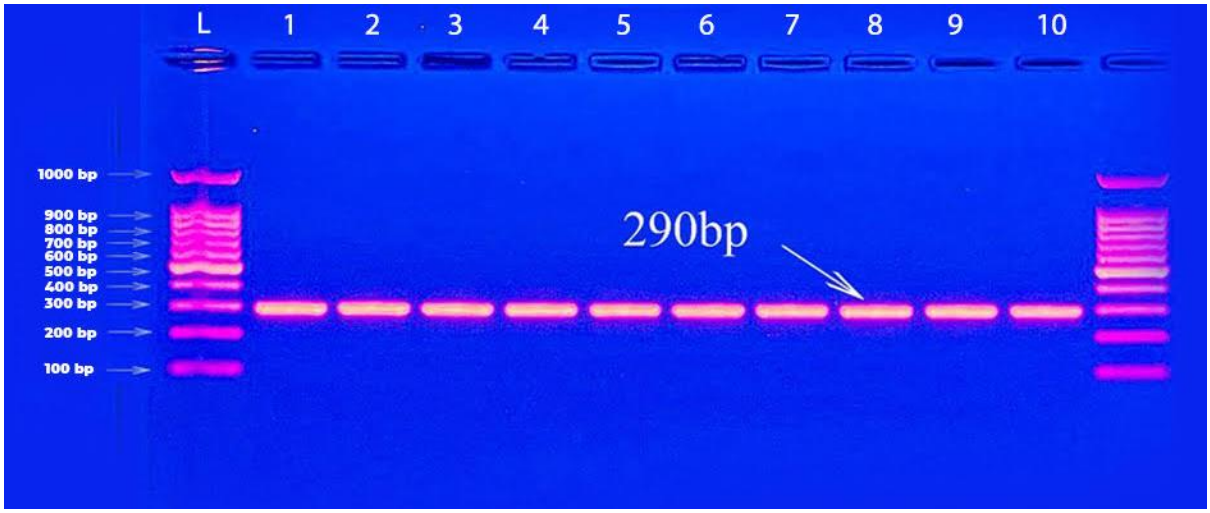

Figure 5: Agarose gel electrophoresis of (LuxS) gene of approximately (290bp), Lanes: L, 100bp DNA ladder; 1 to 10 are number of positive samples.

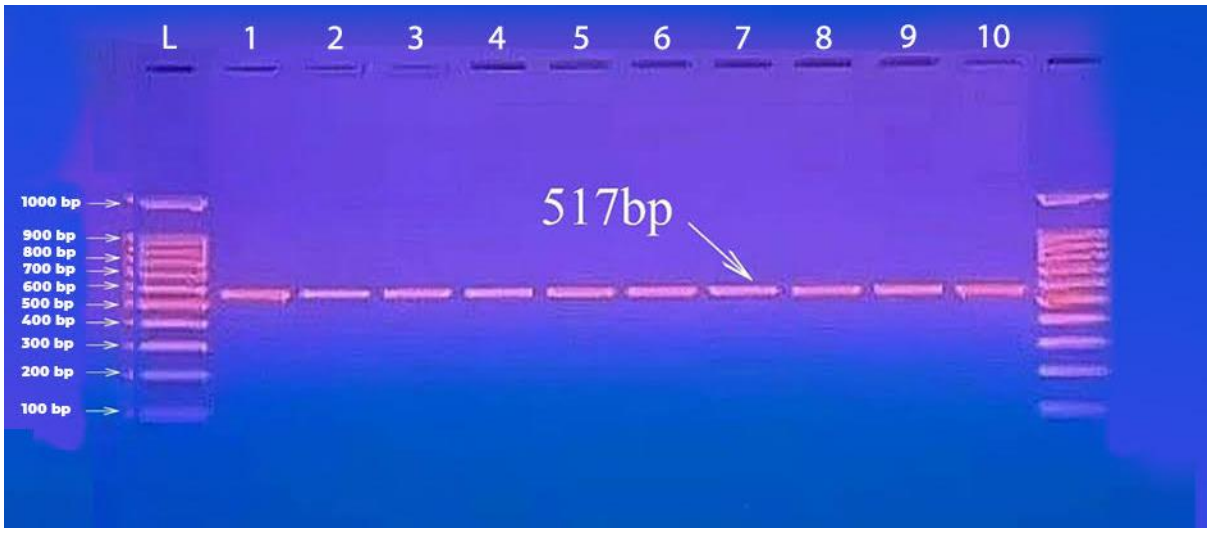

Figure 6: Agarose gel electrophoresis of (UreC) gene of approximately (517bp), Lanes: L, 100bp DNA ladder; 1 to 10 are number of positive samples.

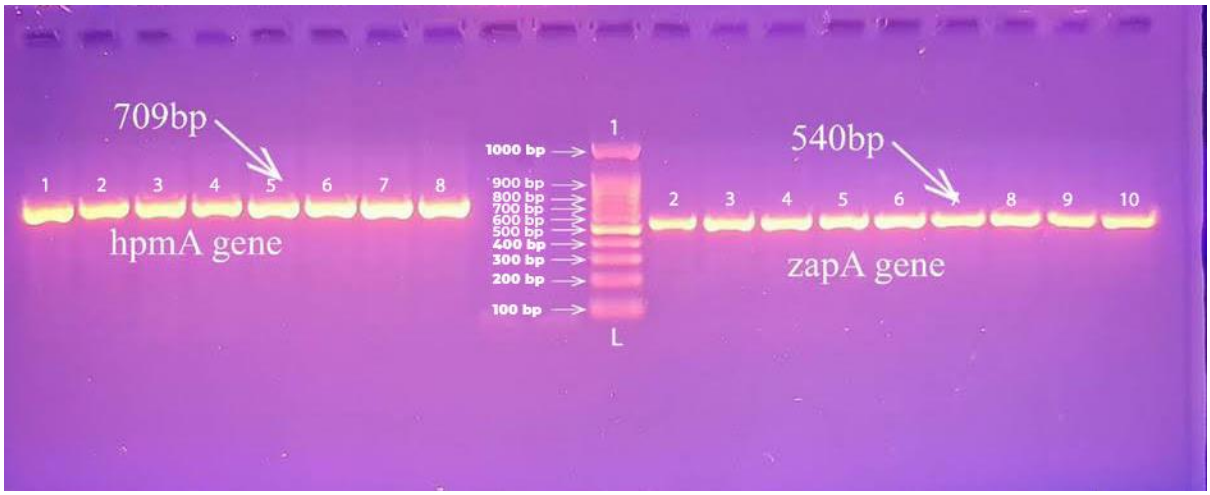

Figure 7: Agarose gel electrophoresis with two set of primers of (HpmA) gene of approximately (709bp) and (ZapA) gene (540bp), Lanes: L, 100bp DNA ladder; 1 to 10 are number of positive samples.

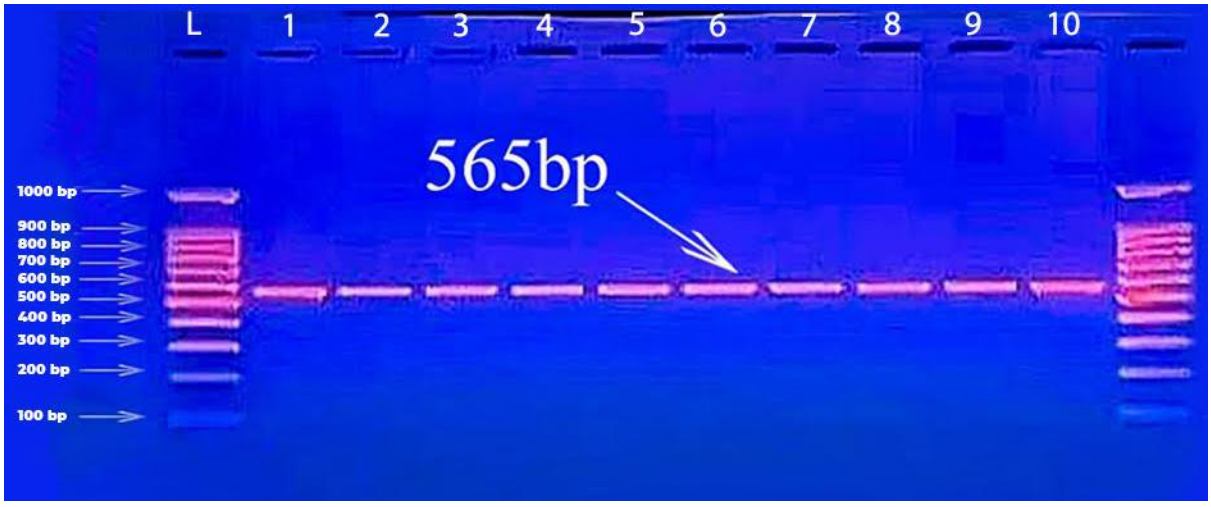

Figure 8: Agarose gel electrophoresis of (MrpA) Gene (565bp), Lanes: L, 100bp DNA ladder; 1 to 10 are number of positive samples.

4 Discussion

Obtained results of Proteus mirabilis isolated from urinary tract infection (UTI) patients in Sulaymaniyah/Iraq, emphasizes several key findings: Due to anatomical and physiological features, (UTI) primarily occur in females. Gender distribution is a reflection of the well-established epidemiological pattern of (UTIs). On account of a number of reasons, including the influence of estrogen on urothelial defense mechanisms and the shorter distance between the urethral opening and the bladder, women are more likely to be susceptible to these infections than men 13, which is the reason that positive P. mirabilis isolates from this study showed (80%) from females and (20%) from males. Similar to another study done in Dohuk/Iraq that showed isolation percentage of (70%) and (30%) among females and males, respectively 14. However, in the current study no statistically significant correlation was found between gender and the incidence of urinary tract infections (UTIs) (Fisher's Exact Test, p = 0.56). Although, Females had a marginally greater prevalence of UTI (92.9%, 65/70) than males (89.4%, 42/47), Ten isolates were identified by evaluating their features on (blood agar, MacConkey agar), P. mirabilis was confirmed through swarming on blood agar and having a fishy odor and smooth pale colonies on MacConkey agar prior to diagnosis with BD Phoenix system and molecular confirmation. Results by BD Phoenix system showed significant regional differences concerning carbapenem by susceptibility to ertapenem (100%) contrasted with a study conducted in Zakho/Iraq which found (88.4%) imipenem resistance among (95) isolates from urine, ear, sputum, burn, wound, and vagina 15. On the other hand another research in Sulaymaniyah/Iraq showed that P. mirabilis was susceptible (100%) to cefepime and imipenem while resistant to all other tested antibiotics 16. Another study in Baghdad/Iraq found (15.3%) of cases were resistant to imipenem 17. Results matched with a study conducted in Duhok/Iraq found (92%) imipenem susceptible and this is similar to current results in which isolates were carbapenem-sensitive which possess a high effectiveness against gram negative bacteria 14 whereas Zakho/Iraq isolates gained remarkable resistance 15. Beta-Lactam resistance patterns showed that all isolates (10/10; 100%) were sensitive to piperacillin-tazobactam resistance which had similar values to the study carried out in Baghdad's city (89.2%) 17. Ampicillin resistance (6/10; 60%) matched with results obtained from Zakho (74.7%) and Baghdad (75%) 15, 17. Eighty percent susceptibility of isolates of this study to advanced cephalosporins (ceftriaxone, cefepime) matched Zakho's (90.3%) 15. When aminoglycoside action wasobserved ; our results showed better amikacin activity which is (90.0%) of isolates were susceptible and (10%) were resistant meanwhile (70.5%) of isolates from Zakho were susceptible 15. Concerning Gentamicin showing, (5/10; 50%) of isolates were susceptible, similar to Zakho's (53.7%) 15, 18. Fluoroquinolone resistance showed (3/10; 30%) resistance to ciprofloxacin and (30%) levofloxacin resistance matched Zakho's (41%), indicating fluoroquinolones' unreliability across this region 15.When comparing virulence genes uniformity, in current study all of the 10 isolates included five virulence genes (UreC, MrpA, ZapA and LuxS) and only two isolates lacked (HpmA). Studies conducted in other regions show more uniformity. A study from Zakho MDR isolates showed high but variable prevalence concerning (ZapA 81%, MrpA 69%) 15. Also, Al-Khalidy's study found moderate uniformity (90%) concerning (LuxS) 18. Sheed Al-Jubouri's study found (LuxS) in (95%) of biofilm producers, (MrpA) in (25%) of biofilm producer isolates 11. Regarding the urease enzyme which is regulated by (UreA, UreB and UreC) genes, as well as (UreD, UreE and UreF) as accessory genes 4, all of the ten isolates were positive for urease gene (UreC). Similar to another study conducted in Baghdad, (9/10; 90%) of isolates were positive for urease 19. Additionally, in the current study biochemical test results for urease activity were positive. In vitro urease hydrolysis urea into NH3 and carbon dioxide, the ammonia production will increase the pH of the medium to alkaline at pH 8.1 which changes the color of the indicator red phenol to pink. While in vivo urease (cytoplasmic nickel metalloenzyme) increases calcium crystals formation and magnesium ammonium phosphate precipitates enabling the development of a crystalline biofilm on the catheter which later shields this pathogen from the host immune response and antibiotics. Also, urease in P. mirabilis significantly increases the formation of kidney stones which obstructs urine drainage and leading to reflux and facilitating the development of infection to pyelonephritis and septicemia 6, 20.Another virulence gene is (MrpA) which encodes for the fimbriae it is crucial for the pathogenicity of P. mirabilis, acting as essential adhesion structures that enable the bacterium to interface with the tissues of its host. The (MrpA) gene in this study was present in all (100%) of the ten isolates, while a study in Zakho reported the prevalence of (69.23%) 15. Another study in Baquba reported (100%) presence of (MrpA) which were collected from 100 samples of different clinical sources (urinary tract infections, wounds, stool) 21. Fimbriae, facilitate P. mirabilis in adhering to and colonizing diverse surfaces within the host, including epithelial cells of the urinary system. Furthermore, fimbriae facilitate the development of crystalline biofilms and encrustation, which are characteristic signs of P. mirabilis associated UTI. The coordinated function of fimbriae in adhesion and biofilm development highlights their importance in the pathogenicity of P. mirabilis 3. The virulence gene (HpmA), which was present in (8/10 ;80%) of the isolates in our study, was present in (9/10; 90%) of the isolates of the study done by Al-hamdani in Baghdad 19. Also 17 out of 20 (85%) isolates were positive for hemolysin (HpmA) in another study conducted in Baghdad to 22. (HpmA) encodes for Serratia-type calcium independent hemolysin, one of several tactics used by P. mirabilis to elude host defenses. This hemolysin demonstrates a wide range of cytolytic activity by lysing both nucleated and non-enucleated red blood cells. The significance of this toxin in the overall pathogenicity of P. mirabilis has been highlighted by research findings that show mutants lacking hemolysin to be less virulent 3. Concerning (ZapA) gene which produces protease enzyme in which the survival and virulence of P. mirabilis particularly in the urinary system, depend on this enzyme. Bacteria encounter formidable host defenses, such as antibodies and antimicrobial peptides, throughout the urinary system, making survival difficult for them. (ZapA) is also known as mirabilysin. It is a powerful

metalloprotease that can break down a wide variety of host proteins in laboratory tests 3. (ZapA), expression stays high at the periphery of a growing swarm colony which was the reason that all of the 10 (100%) P. mirabilis isolates in this study expressed swarming characteristic on blood agar, which agree with a study in Nasiriyah City/Iraq which also reported (100%) positivity for carrying (ZapA) gene 23. While, in another study conducted in Al-Najaf province/Iraq reported that (6/20; 30%) of isolates from different sources possessed (ZapA) gene 3. Regarding (LuxS) gene it encodes the enzyme S-ribosyl-homocysteine lyase, which catalyzes the synthesis of autoinducer-2 (AI-2), a universal quorum-sensing signal that mediates inter-species communication among bacteria. AI-2 production allows bacterial populations to coordinate collective behaviors such as biofilm formation, swarming motility, virulence factor expression, and stress responses18. In P. mirabilis isolated from (UTI), the (LuxS) gene was detected in all of the 10 tested isolates (100 % prevalence), comparing with another study in Baghdad revealed that this gene was present in (22/22; 100%) of the isolates 24. This confirms its widespread presence in clinical strains in this region. By regulating genes involved in adhesion, extracellular enzyme secretion, and motility, this gene contributes to the pathogen's ability to colonize the urinary tract and resist host defenses. Because AI-2 signaling influences multiple pathways, so (LuxS) is considered a potential target for strategies aimed at disrupting quorum sensing and attenuating bacterial virulence 18.

5 Conclusion

The present study offers preliminary molecular and phenotypic characterization of Proteus mirabilis isolates collected from hospitals in Sulaymaniyah. The identification of certain virulence-associated genes, along with the reported antibiotic susceptibility patterns, there was no statistically significant association between gender and the occurrence of urinary tract infections (UTIs). Results enhance the foundational data regarding the genetic and antimicrobial characteristics of these isolates. All Proteus mirabilis isolates demonstrated multidrug resistance (MDR), indicating a potential correlation between MDR and the expression of virulence factors, such as hemolysin synthesis and biofilm formation, among the isolates. The predominance of virulence-associated genes in P. mirabilis isolates from Sulaymaniyah hospitals highlights the importance of molecular diagnostics in understanding pathogenic profiles and guiding infection control and treatment strategies. These results may have potential relevance for guiding infection control and antimicrobial strategies using monitoring and targeted treatments to reduce UTI morbidity. These findings correspond with the rising prevalence of multidrug-resistant P. mirabilis in (UTIs) throughout the Middle East.

6 Limitations of study

This study selected patients diagnosed with UTI from tertiary hospitals in Sulaymaniyah city and did not include hospitals of other levels. The study did not focus on specific age group; it can serve as a reference and represent for not fully specified population. Due to the restricted sample size and descriptive methodology, the results must be regarded with caution. The findings do not provide conclusive determinations about overall virulence potential, regional epidemiological patterns, or clinical significance. Rather, they signify the existence of often documented virulence factors in the examined isolates, aligning with the established role of P. mirabilis as a uropathogen.

7 Recommendations for future studies

Selecting primary and secondary hospitals in various local area and regions in which cover and represent the greatest possible included patients with UTI consequently calculating epidemiologically prevalence of virulence genes. Future investigations should utilize whole-genome sequencing to identify resistance determinants and clonal relationships, establish longitudinal surveillance for monitoring resistance dynamics, and correlate genotypic profiles with clinical outcome data.

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Author's contribution

The author was responsible for the study design, sample collection, laboratory work, data analysis, and manuscript preparation. The supervisor contributed to the study supervision, scientific guidance, data interpretation, and critical revision of the manuscript. Laboratory members and hospital staff assisted in sample processing and technical support during the study.

Conflict of Interest

The author declares no conflicts of interest related to this study. The funders had no role in the study design, data collection, data analysis, manuscript preparation, or the decision to publish this work.

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