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# Detection of β-Lactamase and Virulence-Associated Genes in Multidrug-Resistant Proteus Mirabilis Isolates from Urinary Tract Infections

**Aqeel Shanan Omran**

Education Directorate of Al-Qadisiyah, Ministry of Education, Iraq

**Abstract**

**Introduction:** P. mirabilis is becoming an opportunistic pathogen of significant importance among those causing UTIs. Increased incidence of P. mirabilis strains resistant to multiple antibiotics and exhibiting virulence factors has been reported. Resistance to beta-lactam antibiotics due to the development of beta-lactamase enzymes has created numerous difficulties for UTI treatment. The current research aimed to investigate the antimicrobial sensitivity, phenotypic production of ESBLs, biofilm-forming capacity, hemolytic capability, and molecular identification of resistance- and virulence-associated genes in P. mirabilis strains isolated from UTI cases in Al-Diwaniyah, Iraq.

**Methods:** Urine samples from UTI patients were collected and cultured to isolate the test bacteria. The bacteria were detected using traditional microbiological and biochemical analysis and the VITEK 2 system. Susceptibility tests to different antimicrobials were performed utilizing the Kirby-Bauer disc diffusion technique. The double-disc synergy test accomplished phenotypic determination of ESBL. Biofilm-forming capability and hemolytic activity were analyzed using the Congo Red Agar and Blood Agar media, respectively. Gene detection was performed by PCR targeting the 16S rRNA, blaTEM, blaSHV, blaCTX-M-8, blaCTX-M-9, hpmA, and luxS genes.

**Results:** Multi-drug resistance was detected in P. mirabilis strains against ampicillin, erythromycin, clindamycin, clarithromycin, penicillin G, cephalothin, and cefaclor. Many of the isolates produced ESBLs, formed biofilms, and exhibited hemolytic activity. The PCR results showed the presence of the blaTEM (516 bp), blaSHV (1000 bp), blaCTX-M-8, blaCTX-M-9, hpmA, and luxS genes.

**Conclusion:** The simultaneous presence of multidrug resistance and virulence factors in clinical isolates of P. mirabilis underscores the need for continuous monitoring and preventive measures to prevent the spread of resistance.

**Keywords:** Proteus mirabilis, UTI, β-Lactamase genes, Biofilm formation, Antibiotic resistance

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## Introduction

Urinary tract infections are among the most commonly encountered bacterial infections affecting patients of all ages worldwide. Additionally, these infections are significant for public health because of their high prevalence, tendency to recur, and increasing antibiotic resistance (1). In general, Gram-negative bacteria are the principal causative agents of UTIs, and the major pathogens include members of the Enterobacteriaceae, namely E. coli, K. pneumoniae, and P. mirabilis (2). It is important to note that the latter is considered an opportunistic pathogen in UTIs (3).

First of all, the bacterium P. mirabilis is considered highly motile and a Gram-negative organism characterized by such properties as swarming, urease production, hemolysis, and biofilm formation, making it pathogenic and persistent in the urinary system (4). Moreover, the bacterium can infect not only epithelial tissues but also urinary catheters, causing UTIs primarily in hospitalized or immunocompromised patients (5). In addition, P. mirabilis has many genes involved in biofilm formation, quorum sensing, hemolysin production, tissue invasion, and immune evasion (6).

In recent decades, antimicrobial resistance rates among P. mirabilis isolates have increased in many countries worldwide, posing a therapeutic challenge today (7). One of the main mechanisms of resistance development in Gram-negative bacteria involves β-lactamases, which degrade β-lactam antibiotics, including penicillins, cephalosporins, and monobactams (8). In particular, one should pay attention to Extended Spectrum β-Lactamases (ESBLs), which confer resistance to third-generation cephalosporins and aztreonam (9).

Finally, a range of P. mirabilis strains have already been shown to carry a variety of genes involved in β-lactamase expression, such as blaTEM, blaSHV, and blaCTX-M variants (10). For instance, TEM enzymes are very common β-lactamase variants in Gram-negative bacteria, conferring resistance to ampicillin and other β-lactams (11). SHV enzymes confer resistance to broad-spectrum cephalosporins and are spread horizontally via plasmids (12). Nowadays, CTX-M-type ESBLs are widely used due to their predominance in multidrug-resistant strains (13).

In addition to antimicrobial resistance, virulence-related

*Corresponding Author: Aqeel Shanan Omran, Email: akeelalmyali@gmail.com

genes such as hpmA and luxS are critical to P. mirabilis virulence. Specifically, the former produces a hemolysin that leads to tissue injury and bacterial invasion, while the latter enables quorum sensing and biofilm formation, thereby ensuring bacterial persistence (14). Therefore, the presence of both resistance and virulence factors may significantly increase bacterial survival rates and pathogenicity, as well as the likelihood of treatment failure (15).

Although many scientific studies have addressed antimicrobial resistance among P. mirabilis strains worldwide, data remain limited on the molecular mechanisms underlying ESBL production, biofilm formation, and virulence factors in P. mirabilis strains isolated from UTI patients in Iraq. The present study focuses on antibiotic susceptibility, ESBL production, biofilm formation, and hemolysis, as well as the detection of four selected β-lactamase genes and virulence factors in P. mirabilis UTI isolates.

## Materials and Methods

### *Study Framework and Sample Acquisition*

This investigation will be conducted using a cross-sectional approach in February and March 2025 to evaluate the resistance patterns and virulence factors of Proteus mirabilis among patients with UTIs. For this purpose, approximately 200 urine samples were collected from patients across various clinical departments of Al-Diwaniyah Teaching Hospital and Al-Diwaniyah Maternity and Children Hospital in Al-Diwaniyah Province, Iraq. Urine specimens were aseptically carried to the microbiology laboratory in properly sterilized tubes.

### *Isolation and Identification of Proteus mirabilis*

Procedures used for the isolation and identification of Proteus mirabilis from collected urine samples included inoculating approximately 50 µL of each sample onto MacConkey agar plates and subsequent aerobic incubation at 37°C for 24 hours. The identification of isolates was performed using morphological characteristics, including swarming, non-lactose fermentation, and light-colored colonies. The bacteriological tests included the standard Gram stain and biochemical characterization, which included urease, catalase, oxidase, indole, citrate, gelatin hydrolysis, H2S, MR, and motility tests (16). Isolates will be characterized through the VITEK 2 Compact System (bioMérieux, France).

### *Antimicrobial Susceptibility Testing*

To determine antimicrobial susceptibility profiles, the disk diffusion test was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (17). Using the Kirby-Bauer disk diffusion method, antimicrobial susceptibility patterns of isolated strains to 26 antimicrobial drugs across various categories were determined (Table 1). Zones of inhibition were measured after 18-24 hours of incubation at 37°C, in accordance with CLSI guidelines.

**Table 1.** Antibiotics used for antimicrobial susceptibility testing

| Antibiotic | Symbol | Concentration (µg/disc) |
|---|---|---|
| Ampicillin | AMP | 10 |
| Carbenicillin | CB | 100 |
| Neomycin | N | 30 |
| Gentamicin | GEN | 10 |
| Erythromycin | E | 15 |
| Imipenem | IPM | 10 |
| Clindamycin | CD | 2 |
| Clarithromycin | CLR | 15 |
| Norfloxacin | NX | 10 |
| Amoxicillin/Clavulanic acid | AMC | 30 |
| Chloramphenicol | C | 30 |
| Cefepime | CPM | 30 |
| Streptomycin | S | 10 |
| Tetracycline | TE | 30 |
| Cephalothin | CEP | 30 |
| Azithromycin | AZM | 15 |
| Tobramycin | TOB | 10 |
| Ciprofloxacin | CIP | 5 |
| Aztreonam | AT | 30 |
| Penicillin G | P | 10 units |
| Cefaclor | CF | 30 |
| Ceftazidime | CAZ | 30 |
| Nalidixic acid | NA | 30 |
| Ceftriaxone | CTR | 30 |
| Cefotaxime | CTX | 30 |
| Fosfomycin | FO | 200 |

### *Phenotypic Detection of ESBLs Production*

ESBL generation was identified using the double-disk synergy test (DDST). The assay was performed according to a previously published method (18). Briefly, bacterial inoculations with 0.5 McFarland were done on Mueller-Hinton Agar. Following this, amoxicillin-clavulanic acid disks (30 µg) were put on the centers of plates, while cefotaxime, ceftazidime, and aztreonam disks were set around 20-30 mm from the centers. Incubation was done at 37°C for 18-24 hours. Any expansion of the inhibition zone against the clavulanic acid disk was deemed a positive indication of ESBL production.

### *Biofilm Formation Detection*

Biofilm formation was performed using the Congo red agar method. Bacterial isolates that formed biofilms produced dark, crystalline colonies after incubation at 37°C for 24-48 hours, whereas those lacking biofilm formation became pinkish to reddish.

### *Hemolysis Detection*

Hemolysis was identified using the ability of bacterial isolates to grow on blood agar plates with 5% sheep blood. The blood agar plates were incubated at 37°C for 24 hours

under aerobic conditions. Clear halos around bacterial colonies detected Hemolysin.

### *DNA Extraction*

Genomic DNA was extracted from each bacterial isolate using the Bioneer genomic DNA extraction kit (Bioneer, Korea) following the manufacturer's guidelines for Gram-negative bacteria. The concentrations of genomic DNA samples were examined before conducting molecular analysis.

### *PCR Amplification of Resistance/Virulence Genes*

Conventional PCR was performed for the molecular detection of 16S rRNA, blaTEM, blaSHV, blaCTX-M-8, blaCTX-M-9, hpmA, and luxS, the resistance and virulence genes (Table 2). The ingredients of the PCR reaction (25 µL final volume) were 12.5 µL GoTaq® Green Master Mix, 1 µL template DNA, 1 µL each of forward and reverse primers (10 pmol/µL), and nuclease-free water.

The conditions for the PCR amplification process were as follows: denaturation at 94°C for 10 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 3 minutes, and final extension at 72°C for 10 minutes. The PCR amplification of blaSHV was carried out under identical conditions, except for a 5-minute denaturation step at 94°C.

Agarose gel electrophoresis of the amplified DNA fragments was performed on a 2% agarose gel with ethidium bromide staining and viewed under a UV light source. A 100bp DNA ladder was used.

### *Statistical Analysis*

Data analysis was done using MS Excel 2019. Data presentation was done using percentages.

## Results

### *Isolation and Identification of Proteus Mirabilis*

Urinary samples obtained from individuals with urinary tract infections were used to isolate pathogenic bacteria using selective and differential culture techniques. Isolates confirmed as Proteus mirabilis exhibited characteristic features, including pale, non-lactose-fermenting colonies on MacConkey agar plates and high swarming motility on blood agar plates (Figure 1). The colonies appeared smooth, circular, wet, and highly mobile, characteristic of P. mirabilis. Under Gram staining reaction, Gram-negative bacilli were observed. Biochemical tests confirmed that the isolates were positive for catalase, gelatin liquefaction, urease, methyl red, motility, and hydrogen sulfide reactions; however, the oxidase and indole reactions were negative. The VITEK 2 automated identification system confirmed the isolates.

### *Pattern of Antibiotic Sensitivity of Proteus mirabilis Isolates*

From the results of the antibacterial sensitivity test, considerable variability in multidrug resistance among P. mirabilis isolates was observed against various widely used antimicrobial agents. Complete resistance was observed across all strains to ampicillin (100%), erythromycin (100%), clindamycin (100%), and clarithromycin (100%), penicillin G (100%), cephalothin (100%), and cefaclor (100%). Some resistance was observed against third-generation cephalosporins, including ceftriaxone (85%), cefotaxime (75%), and ceftazidime (60%). Besides, considerable resistance was observed in the aminoglycoside and other antibiotic groups. On the contrary, most of the isolates were sensitive to imipenem, norfloxacin, ciprofloxacin, cefepime, and tobramycin. Various other antibiotics also showed different levels of sensitivity against the isolates (Table 3).

### *Detection of ESBL-Producing Bacteria through Phenotypic Assay*

The double-disk synergy test (DDST) was applied to detect the ability of P. mirabilis isolates to produce ESBL enzymes (Figure 2 and 3). ESBL enzymes were produced by 70% (n = 7) of P. mirabilis isolates. An increased zone of inhibition was noted around cephalosporin disks when combined with amoxicillin/clavulanic acid, signifying that ESBL enzymes are present. The phenotypic identification of ESBL-producing organisms corroborates the molecular assay results on the presence of β-lactamase genes and

**Table 2.** Primers for the amplification of resistance and virulence genes

| Gene | Primer sequence (5′–3′) | Product size (bp) | Reference |
|---|---|---|---|
| 16S rRNA | F: AGAGTTTGATCCTGGCTCAG<br>R: ACGGCTACCTTGTTACGACTT | 1500 | (19) |
| blaSHV | F: CGCCGGGTTATTCTTATTTGTCGC<br>R: TCTTTCCGATGCCGCCGCCAGTCA | 1000 | (15) |
| blaTEM | F: ATGAGTATTCAACATTTCCG<br>R: CTGACAGTTACCAATGCTTA | 516 | (16) |
| blaCTX-M-8 | F: AGCAAAGTGAAACGCAAAAG<br>R: TCATTCGTCGTACCATAATC | 400 | Primer-BLAST design |
| blaCTX-M-9 | F: CGCTTTATGCGCAGACGA<br>R: GATTCTCGCCGCTGAAGC | 500 | Primer-BLAST design |
| hpmA | F: AGGTGCTAAACTGCATGCGA<br>R: ACAAAAGCACCTTGGTTGCC | 270 | Primer-BLAST design |
| luxS | F: ACGTATGTCTGCACCTGCG<br>R: CCATAGCTGCCTTCCATGCA | 290 | Primer-BLAST design |

Primers for blaCTX-M-8, blaCTX-M-9, hpmA, and luxS genes were developed using the NCBI Primer-BLAST tool from the conserved sequences of the genes obtained from the GenBank database.

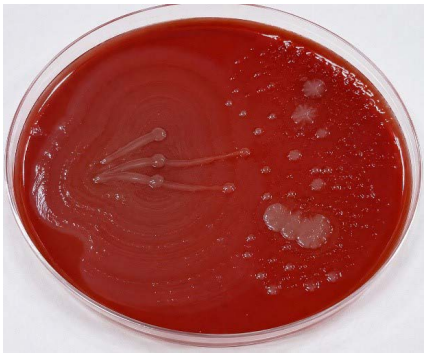

**Figure 1.** Culture and morphological properties of Proteus mirabilis colonies isolated on selective bacteriological medium, displaying typical swarming ability and morphological colony traits for identifying bacteria

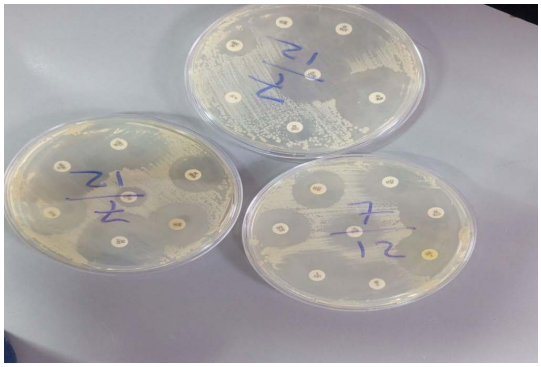

**Figure 2.** Susceptibility patterns of P. mirabilis to various antimicrobial drugs utilizing the Kirby-Bauer method on Mueller-Hinton agar

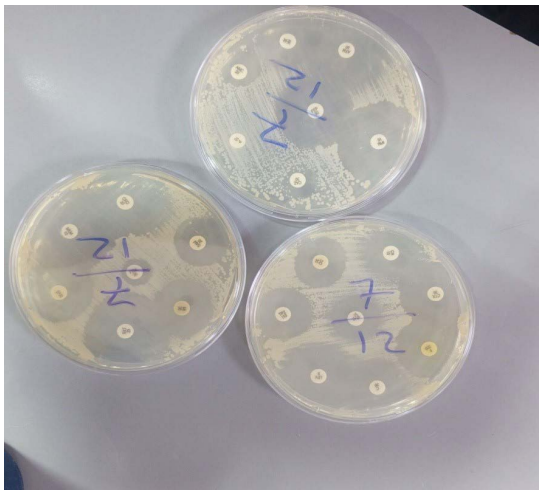

**Figure 3.** Detection of ESBL-producing Proteus mirabilis isolates phenotypically through the double disk synergy test (DDST), showing synergistic enhancement of zones of inhibition among β-lactam antibiotics and disks containing clavulanic acid

indicates a widespread pattern of resistance to β-lactam antibiotics. ESBL-producing bacteria are clinically important because of their resistance to third-generation cephalosporins and other β-lactams, which are routinely used to treat UTIs (Table 4 and 5).

### *Biofilm Production in Proteus mirabilis Isolates*

Some P. mirabilis isolates were shown to produce biofilms on Congo red medium. Biofilm formation occurred in eight of the ten isolates studied (80%). The positive

**Table 3.** Antibiotic resistance profile

| Antibiotic | Resistance (%) |
|---|---|
| Ampicillin | 100 |
| Erythromycin | 100 |
| Clindamycin | 100 |
| Clarithromycin | 100 |
| Penicillin G | 100 |
| Cephalothin | 100 |
| Cefaclor | 100 |
| Ceftriaxone | 85 |
| Cefotaxime | 75 |
| Ceftazidime | 60 |
| Cefoperazone | 45 |

**Table 4.** Prevalence of virulence and resistance phenotype among Proteus mirabilis isolates

| Phenotypic characteristic | Positive isolates n (%) |
|---|---|
| ESBL production | 7 (70%) |
| Biofilm formation | 8 (80%) |
| Hemolytic activity | 8 (80%) |

**Table 5.** Occurrence of resistance- and virulence-associated genes among P. mirabilis isolates

| Gene | Positive isolates n (%) | Product size (bp) |
|---|---|---|
| blaTEM | 6 (60%) | 516 |
| blaSHV | 4 (40%) | 1000 |
| blaCTX-M-8 | 2 (20%) | 400 |
| blaCTX-M-9 | 1 (10%) | 500 |
| hpmA | 8 (80%) | 270 |
| luxS | 7 (70%) | 290 |

isolates produced dark black colonies with a crystalline appearance, indicating extracellular polysaccharide production and biofilm formation (Figure 4). It is important to note that biofilm formation is an essential virulence trait because it contributes to persistence, colonization of tissues and devices within the urinary tract, and greater resistance to host defense mechanisms and antibiotic treatment.

### *Hemolysin Production by Proteus mirabilis*

Hemolysis was observed in 8 of 10 P. mirabilis isolates (80%) grown on blood agar plates. The isolates were found to produce clear areas of hemolysis around the colonies, resulting from hemolysin enzymes that lyse red blood cells. Hemolysin production is a virulence factor associated with P. mirabilis pathogenicity. It enhances virulence and causes increased severity of urinary tract infections by promoting tissue destruction and invasion (Figure 5).

### *Resistance and Virulence Factor Detection in Proteus mirabilis via Molecular Approaches*

#### *Detection of Resistance and Virulence Genes by Molecular Techniques*

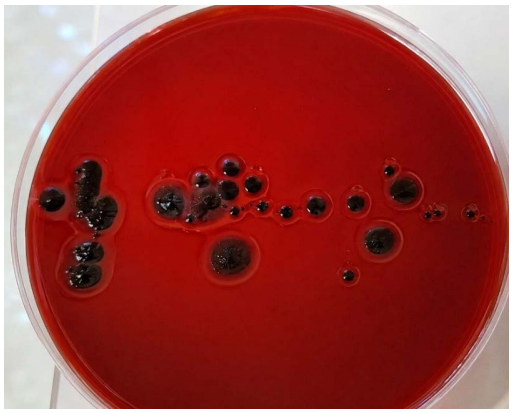

**Figure 4.** Biofilm formation by isolates of Proteus mirabilis on Congo red agar, with black colonies indicating biofilm formation

**Figure 5.** Hemolytic nature of Proteus mirabilis isolates, as evidenced by clearing zones indicating destruction of red blood cells due to bacterial growth, indicating positive hemolysin activity

PCR Amplification of 16S rRNA Gene for Molecular Identification of P. mirabilis isolates

Molecular identification of all P. mirabilis isolates was successfully determined via PCR amplification of the 16S rRNA gene. Unique amplification bands of approximately 1500 bp were observed in all isolates examined. This indicates successful amplification of the bacterial housekeeping gene, thereby confirming their identification at the molecular level (Figure 6).

### *Detection of the blaTEM Gene*

Analysis of PCR amplification demonstrated that the blaTEM gene was present in 6 of 10 P. mirabilis isolates (60%). Positive isolates showed a clear amplification band of about 516 bp, whereas the other isolates did not show any PCR amplification product. This is because the prevalence of the blaTEM gene is high, suggesting the widespread distribution of TEM β-lactamases among the isolates and explaining their high resistance to β-lactams such as penicillins and cephalosporins (Figure 7).

### *Detection of blaSHV Gene*

Of the 10 P. mirabilis isolates tested, 4 (40%) carried the blaSHV gene. The positive strains produced bands of approximately 1000 bp, while the others did not produce any bands. Therefore, the detection of SHV-type β-lactamases confirms the prevalence of ESBL resistance genes among clinical P. mirabilis isolates (Figure 8).

### *Detection of blaCTX-M-8 Gene*

Based on PCR results, the blaCTX-M-8 gene was detected in 2 of 10 Proteus mirabilis strains (20%). Positive amplification products at around 400 bp were observed in 2 strains. It is clinically important to identify β-lactamase CTX-M types due to their resistance to third-generation cephalosporins (Figure 9).

### *Identification of the blaCTX-M-9 Gene*

A successful amplification of the blaCTX-M-9 gene was observed in one out of ten isolated strains of P. mirabilis (10%). Even though the distribution rate of the blaCTX-M-9 gene is lower than that of blaTEM and blaSHV, the presence of this gene in the isolates indicates the presence of various CTX-M β-lactamase enzymes. Amplification of this gene may indicate resistance to cephalosporin-type antibiotics (Figure 10).

### *Detection of the hpmA Gene*

The hpmA gene, associated with the ability to produce hemolysin, was found to be present in 8 out of 10 isolated strains of P. mirabilis (80%). Most of the isolates had positive amplification bands at ~270 bp. A high abundance of the hpmA gene in isolates indicates high virulence potential and correlates with their hemolytic nature (Figure 11).

### *luxS Gene Detection*

PCR amplification revealed that the luxS gene was present in 7 of 10 Proteus mirabilis isolates (70%). The *luxS* gene is involved in quorum sensing and biofilm formation, processes that contribute to bacterial survival, colonization, and increased antibiotic resistance. This high detection rate of luxS genes confirms the biofilm formation results described in this experiment (Figure 12).

## Discussion

Urinary tract infections (UTIs) remain frequent and are associated with multidrug-resistant Gram-negative bacteria (20). In this study, Proteus mirabilis was found to be one of the most common uropathogens isolated from urine cultures of patients with UTIs. The isolates showed typical motility on blood agar and non-lactose fermentation on MacConkey agar plates, which are used for preliminary characterization of this organism (21). Cultural and biochemical traits similar to those described above have also been documented in P. mirabilis isolated from UTIs (22).

The findings of the antimicrobial susceptibility test conducted in this work revealed a considerably high prevalence of multidrug resistance among the tested P. mirabilis strains. These strains demonstrated resistance to a variety of antimicrobials, including β-lactams (ampicillin), macrolides (erythromycin, clindamycin, clarithromycin), and cephalosporins (cephalothin, cefaclor, ceftriaxone, cefotaxime, ceftazidime). High

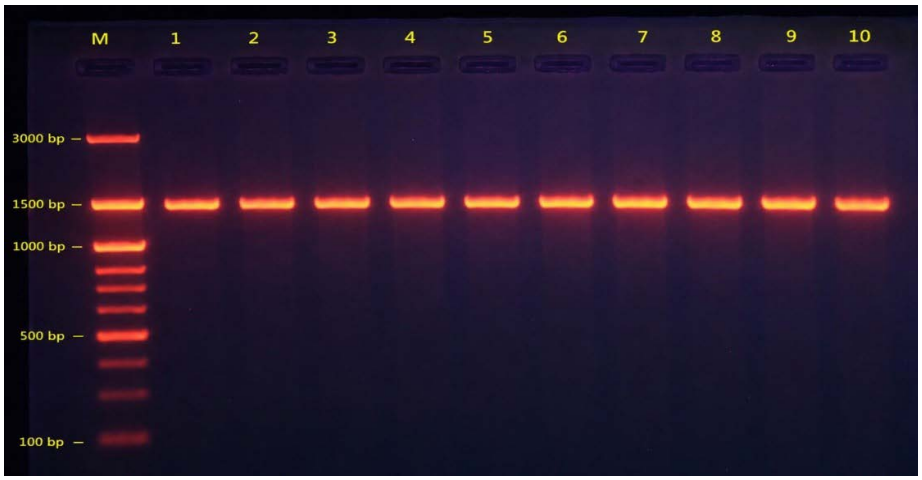


**Figure 6.** Gel electrophoresis on agarose gel showing amplification of the 16S rRNA gene (1500 bp) in the isolate of Proteus mirabilis. Lane M shows the 100 bp DNA marker; lanes 1 to 10 show the 1500 bp amplified product

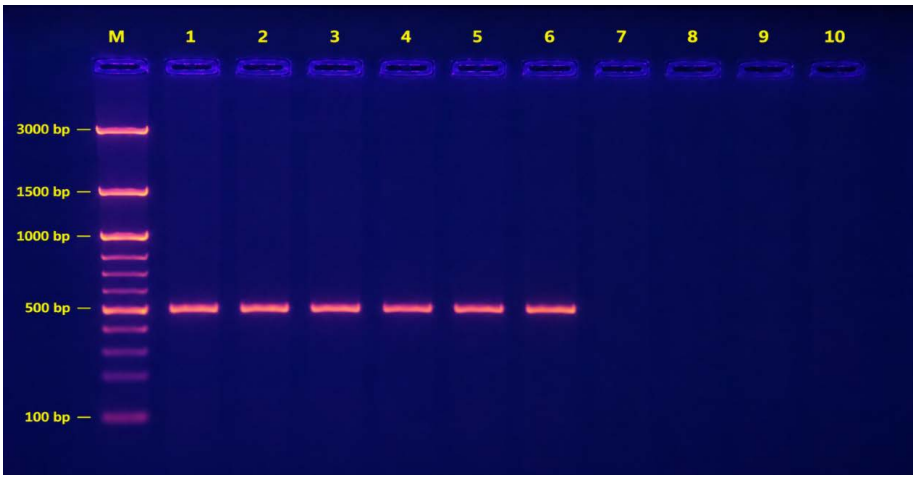


**Figure 7.** Agarose gel electrophoresis demonstrating amplification of the blaTEM gene (approximately 516 bp) by PCR from P. mirabilis isolates. The M lane is the DNA marker lane; lanes 1-6 show positive amplification, while lanes 7-10 show negative amplification

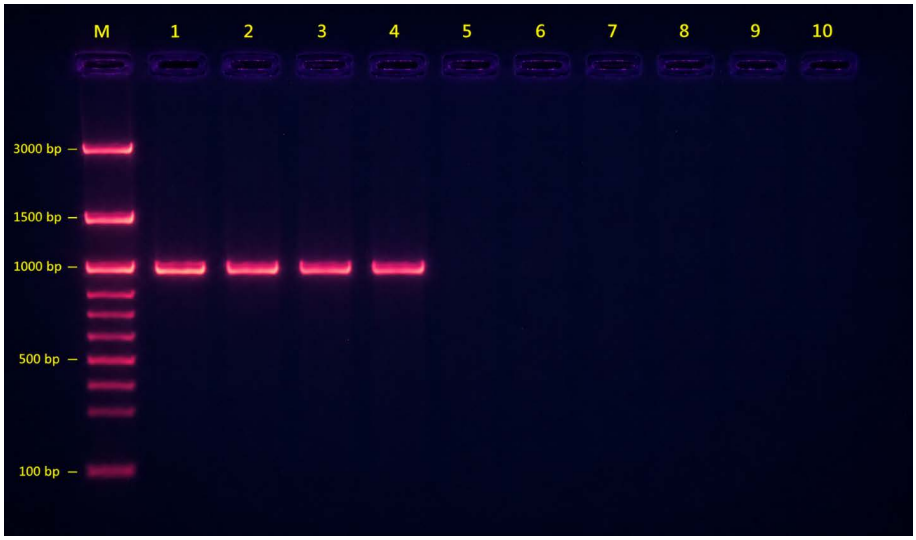


**Figure 8.** Agarose Gel Electrophoresis Showing PCR Amplification of blaSHV Gene (~1000 bp) in Proteus Mirabilis Strains. Lane M is the DNA Marker; Lanes 1 to 4 represent positive amplification results, whereas lanes 5 to 10 show negative amplifications

resistance to third-generation cephalosporins is consistent with data from other studies, which have shown a growing trend in β-lactam and cephalosporin resistance in P. mirabilis (23, 24). Overuse and improper use of antibiotics may be regarded as one of the major causes of the emergence of antibiotic resistance through selective pressure (25).

On the contrary, most isolates retained susceptibility

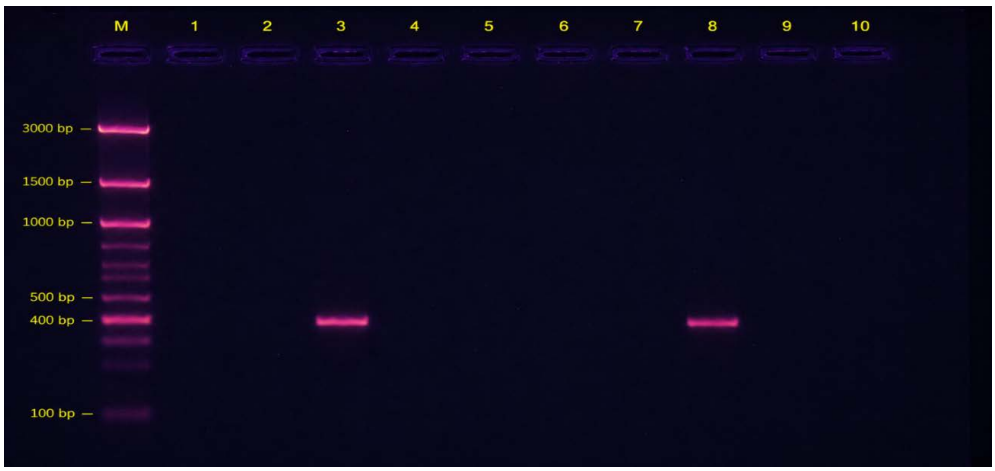


**Figure 9.** Agarose gel electrophoresis results for the amplification of the blaCTX-M-8 gene (~400 bp) in Proteus mirabilis isolates. "M" is the DNA marker; "3" and "8" are positive amplification bands at 400 bp, while all other bands are negative amplifications

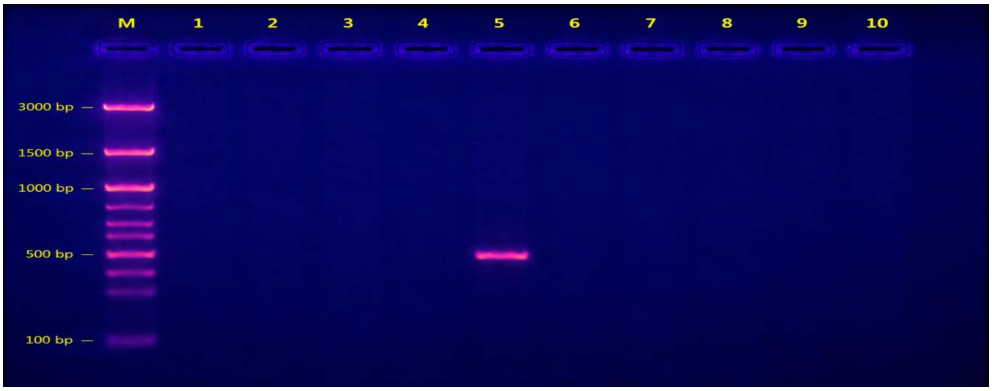


**Figure 10.** Agarose gel electrophoresis showing PCR amplification of the blaCTX-M-9 gene (~500 bp) in isolates of P. mirabilis. In lane M, there is a DNA marker; in lane 5, a positive band is observed at ~500 bp, and all other lanes show negative amplification

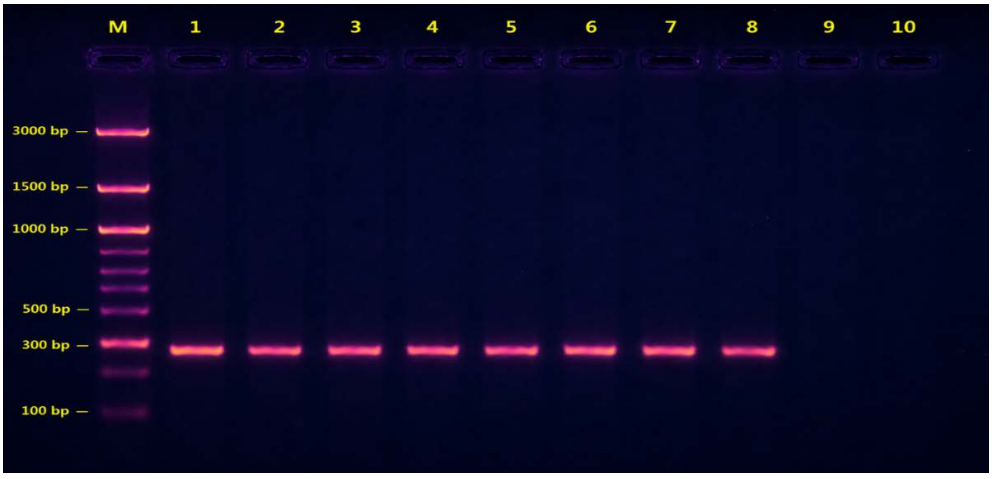


**Figure 11.** Agarose gel electrophoresis for PCR amplification of the hpmA gene (~270 bp) in Proteus mirabilis. M denotes the DNA marker lane, whereas lanes 1-8 correspond to amplification product detection, while lanes 9-10 show no amplification

to imipenem and norfloxacin. Such results are consistent with several earlier studies in which carbapenems have shown high activity against multidrug-resistant P. mirabilis isolates (26). The retention of sensitivity to imipenem can be explained by the broad spectrum of this antibiotic and the low susceptibility of β-lactamase enzymes to its action. Nevertheless, there should be no doubt about the growing prevalence of carbapenem-resistant P. mirabilis strains worldwide (27).

The double-disk synergy test was performed to confirm ESBL production in P. mirabilis isolates obtained from patients included in the current study. The role of ESBLs in the development of resistance mechanisms in Gram-negative bacteria can be explained by their high ability to hydrolyze extended-spectrum cephalosporins, leading to resistance to almost all types of β-lactam antibiotics (28). Information on the frequency of ESBL-positive P. mirabilis strains among patients with UTIs and hospital-

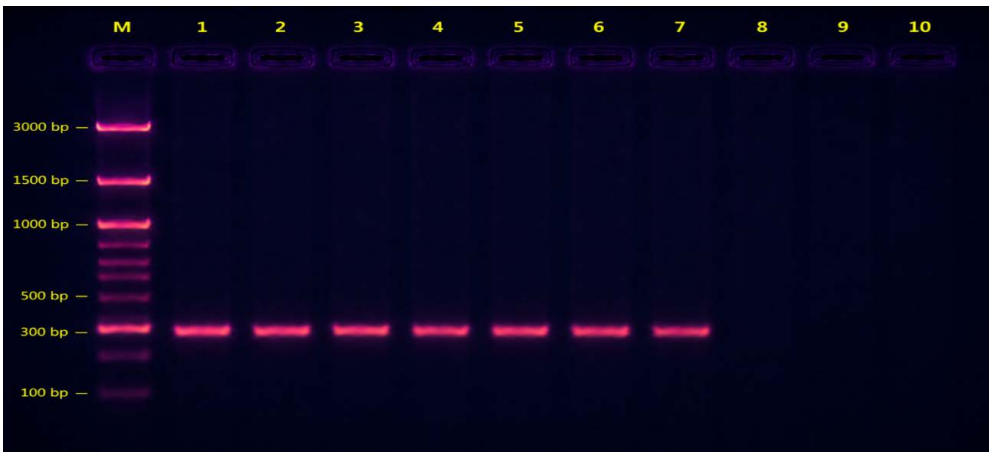


**Figure 12.** Agarose gel electrophoresis showing PCR amplification of the luxS gene (approximately 290 bp) from P. mirabilis isolates. M represents the DNA molecular weight marker, lanes 1–7 represent positive PCR products, while lanes 8–10 indicate negative PCR products

acquired infections was also previously collected (29). Additionally, the presence of ESBL-associated infections is likely to reduce the efficacy of certain β-lactam antibiotics (30).

This research also demonstrated that some of the isolates analyzed produced biofilm and hemolysin, both important bacterial virulence factors. Enhanced adherence to epithelial surfaces and catheters, as well as increased resistance to immune defense mechanisms and antibacterial drugs, are among the most important advantages of biofilms (31). Moreover, the contribution of hemolysin to bacterial invasion and virulence can be explained by its capability to damage tissues, facilitate nutrient intake, and promote bacterial invasion (32). Similar results were found in several earlier studies, in which biofilm formation and hemolysis were identified as major virulence factors associated with P. mirabilis pathogenicity (33).

Amplification of the 16S rRNA gene was useful in confirming that all isolates were P. mirabilis. In fact, the 16S rRNA gene is one of the most widely used methods for identifying bacterial species because of its high conservation and similarity among bacterial families (34). From the current experiment, it was clear that the specific PCR technique helped achieve amplification of the 16S rRNA gene in all isolates.

It was also noted that the blaTEM gene was prevalent in 60% of the isolates. The TEM type of β-lactamase is the predominant ESBL among Gram-negative bacteria resistant to penicillins and cephalosporins (35). Similarly, the prevalence of blaTEM genes observed in this study was comparable to the prevalence rates reported in prior research conducted in different geographic areas (36). The spread of the blaTEM gene appears to be associated with lateral gene transfer via plasmid-mediated mechanisms (37).

In this study, we also detected blaSHV genes in 40% of the analyzed isolates. SHV-type β-lactamases are frequently encountered in hospital infections caused by Enterobacteriaceae and have been associated with wide geographical diversity (38). The prevalence of blaSHV genes has also varied greatly across geographical regions and associated epidemiological conditions. For example, different prevalence rates have been identified previously in various Asian and Middle Eastern countries (39).

Lower prevalence rates of blaCTX-M-8 and blaCTX-M-9 were detected. The CTX-M enzymes have emerged as predominant ESBLs in recent years and were mostly responsible for multidrug resistance to cephalosporins (40). While the prevalence rates obtained in this study were lower than those for blaTEM and blaSHV, detection of blaCTX-M genes is important given their rapid dissemination and growing prevalence worldwide (41).

Moreover, some virulence-related genes, namely hpmA and luxS, were detected in P. mirabilis isolates. The luxS gene is important for regulating biofilm formation, while hpmA encodes a hemolysin (42,43). Based on such findings, it is reasonable to suppose that the hpmA gene was responsible for hemolytic capability and the presence of luxS for biofilm formation.

Overall, the combination of antimicrobial resistance and virulence in P. mirabilis is an important public health concern that can contribute to enhanced survival, increased virulence, recurrent infections, and reduced antibiotic efficacy (44). Therefore, the increasing prevalence of multidrug-resistant and highly virulent P. mirabilis strains requires immediate molecular monitoring, implementation of appropriate antibiotic-use programs, and strict infection-control interventions to reduce further dissemination of these pathogens (45-48).

## Conclusion

This study shows that P. mirabilis isolated from urine cultures of UTI patients harbor numerous antimicrobial resistance genes and virulence determinants, which could increase their pathogenicity. The tested organisms were extensively multidrug-resistant, including to β-lactams and cephalosporins. Phenotypic analysis showed that some isolates produced ESBLs, a finding later confirmed by molecular investigations. Several blaTEM, blaSHV, blaCTX-M-8, and blaCTX-M-9 genes were detected. Moreover, the presence of virulence-related genes, such

as hpmA and luxS, was confirmed in some isolates. The presence of both resistance and virulence genes in P. mirabilis isolates could significantly enhance their pathogenicity and colonization abilities, thereby causing frequent relapse and decreased treatment efficacy.

## Competing Interests

There is no competing interest on the part of the author regarding publishing this study.

## Data Availability Statement

Datasets that were used and analyzed in this current research are accessible to the author on reasonable requests.

## Ethical Approval

The experimental protocol has been approved by the appropriate ethics committee of the Education Directorate of Al-Qadisiyah and has been carried out in accordance with institutional ethical guidelines. Verbal consent was gained from all subjects before sampling.

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## References

1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015;13(5):269-84. doi:10.1038/nrmicro3432
2. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med 2002;113 Suppl 1A:5S-13S. doi:10.1016/s0002-9343(02)01054-9
3. Schaffer JN, Pearson MM. *Proteus mirabilis* and urinary tract infections. In: Mulvey MA, Klumpp DJ, Stapleton AE, eds. Urinary Tract Infections: Molecular Pathogenesis and Clinical Management. John Wiley & Sons; 2017. p. 383-433. doi:10.1128/9781555817404.ch17
4. Armbruster CE, Mobley HL. Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. Nat Rev Microbiol 2012;10(11):743-54. doi:10.1038/nrmicro2890
5. Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. Clin Microbiol Rev 2008;21(1):26-59. doi:10.1128/cmr.00019-07
6. Wasfi R, Hamed SM, Amer MA, Fahmy LI. *Proteus mirabilis* biofilm: development and therapeutic strategies. Front Cell Infect Microbiol 2020;10:414. doi:10.3389/fcimb.2020.00414
7. Ali HH, Yousif MG. Detection of some virulence factors genes of *Proteus mirablis* that isolated from urinary tract infection. Int J Adv Res 2015;3(1):156-63.
8. Bush K, Bradford PA. β-lactams and β-lactamase inhibitors: an overview. Cold Spring Harb Perspect Med 2016;6(8):a025247. doi:10.1101/cshperspect.a025247
9. Rawat D, Nair D. Extended-spectrum β-lactamases in gram-negative bacteria. J Glob Infect Dis 2010;2(3):263-74. doi:10.4103/0974-777x.68531
10. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev 2005;18(4):657-86. doi:10.1128/cmr.18.4.657-686.2005
11. Cantón R, Coque TM. The CTX-M β-lactamase pandemic. Curr Opin Microbiol 2006;9(5):466-75. doi:10.1016/j.mib.2006.08.011
12. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum β-lactamases: types, epidemiology and treatment. Saudi J Biol Sci 2015;22(1):90-101. doi:10.1016/j.sjbs.2014.08.002
13. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother 2017;72(8):2145-55. doi:10.1093/jac/dkx146
14. Liaw SJ, Lai HC, Wang WB. Modulation of swarming and virulence by fatty acids through the RsbA protein in *Proteus mirabilis*. Infect Immun 2004;72(12):6836-45. doi:10.1128/iai.72.12.6836-6845.2004
15. Li Y, Yin M, Fang C, Fu Y, Dai X, Zeng W, et al. Genetic analysis of resistance and virulence characteristics of clinical multidrug-resistant *Proteus mirabilis* isolates. Front Cell Infect Microbiol 2023;13:1229194. doi:10.3389/fcimb.2023.1229194
16. Colom K, Pérez J, Alonso R, Fernández-Aranguiz A, Lariño E, Cisterna R. Simple and reliable multiplex PCR assay for detection of blaTEM, blaSHV and blaOXA-1 genes in *Enterobacteriaceae*. FEMS Microbiol Lett 2003;223(2):147-51. doi:10.1016/s0378-1097(03)00306-9
17. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing (CLSI supplement M100). 34th ed. Wayne, PA: CLSI; 2024.
18. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev Infect Dis 1988;10(4):867-78. doi:10.1093/clinids/10.4.867
19. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl Environ Microbiol 2008;74(8):2461-70. doi:10.1128/aem.02272-07
20. Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. Ther Adv Urol 2019;11:1756287219832172. doi:10.1177/1756287219832172
21. O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. Clin Microbiol Rev 2000;13(4):534-46. doi:10.1128/cmr.13.4.534
22. Drzewiecka D. Significance and roles of *Proteus* spp. bacteria in natural environments. Microb Ecol 2016;72(4):741-58. doi:10.1007/s00248-015-0720-6
23. Girlich D, Bonnin RA, Dortet L, Naas T. Genetics of acquired antibiotic resistance genes in *Proteus* spp. Front Microbiol 2020;11:256. doi:10.3389/fmicb.2020.00256
24. Hafiz TA, Alghamdi GS, Alkudmani ZS, Alyami AS, AlMazyed A, Alhumaidan OS, et al. Multidrug-resistant *Proteus mirabilis* infections and clinical outcome at tertiary hospital in Riyadh, Saudi Arabia. Infect Drug Resist 2024;17:571-81. doi:10.2147/idr.S448335
25. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T 2015;40(4):277-83.
26. Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC Antimicrob Resist 2021;3(3):dlab092. doi:10.1093/jacamr/dlab092
27. Fritzenwanker M, Falgenhauer J, Hain T, Imirzalioglu C, Chakraborty T, Yao Y. The Detection of Extensively Drug-Resistant Proteus mirabilis Strains Harboring Both VIM-4 and VIM-75 Metallo-β-Lactamases from Patients in Germany. Microorganisms 2025;13(2):266. doi:10.3390/microorganisms13020266
28. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001;14(4):933-51, table of contents. doi:10.1128/cmr.14.4.933-951.2001
29. Mirzaei A, Habibi M, Bouzari S, Asadi Karam MR. Characterization of antibiotic-susceptibility patterns, virulence factor profiles and clonal relatedness in *Proteus mirabilis* isolates from patients with urinary tract infection in Iran. Infect

Drug Resist 2019;12:3967-79. doi:10.2147/idr.S230303
30. Tamma PD, Rodriguez-Bano J. The use of noncarbapenem β-lactams for the treatment of extended-spectrum β-lactamase infections. Clin Infect Dis 2017;64(7):972-80. doi:10.1093/cid/cix034
31. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2004;2(2):95-108. doi:10.1038/nrmicro821
32. Coker C, Poore CA, Li X, Mobley HL. Pathogenesis of *Proteus mirabilis* urinary tract infection. Microbes Infect 2000;2(12):1497-505. doi:10.1016/s1286-4579(00)01304-6
33. Yang A, Tian Y, Li X. Unveiling the hidden arsenal: new insights into *Proteus mirabilis* virulence in UTIs. Front Cell Infect Microbiol 2024;14:1465460. doi:10.3389/fcimb.2024.1465460
34. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol 2007;45(9):2761-4. doi:10.1128/jcm.01228-07
35. Dziri R, Talmoudi A, Barguellil F, Ouzari HI, El Asli MS, Klibi N. Huge diversity of TEM and SHV β-lactamases types among CTX-M-15-producing *Enterobacteriaceae* species in Tunisia. Microb Drug Resist 2019;25(8):1149-54. doi:10.1089/mdr.2018.0445
36. Ibrahim MA, Faisal RM. Molecular characterization of antibiotic resistance and virulence genes on plasmids of *Proteus mirabilis* isolated from urine samples of Hospitals in Mosul city, Iraq. J Appl Nat Sci 2024;16(2):830-41. doi:10.31018/jans.v16i2.5526
37. Carattoli A. Resistance plasmid families in *Enterobacteriaceae*. Antimicrob Agents Chemother 2009;53(6):2227-38. doi:10.1128/aac.01707-08
38. Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC-type β-lactamases. Antimicrob Agents Chemother 2002;46(1):1-11. doi:10.1128/aac.46.1.1-11.2002
39. Hussain A, Shaik S, Ranjan A, Nandanwar N, Tiwari SK, Majid M, et al. Risk of transmission of antimicrobial resistant *Escherichia coli* from commercial broiler and free-range retail chicken in India. Front Microbiol 2017;8:2120. doi:10.3389/fmicb.2017.02120
40. Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 2004;48(1):1-14. doi:10.1128/aac.48.1.1-14.2004
41. Zhao WH, Hu ZQ. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in gram-negative bacteria. Crit Rev Microbiol 2013;39(1):79-101. doi:10.3109/1040841x.2012.691460
42. Alamuri P, Eaton KA, Himpsl SD, Smith SN, Mobley HL. Vaccination with *Proteus* toxic agglutinin, a hemolysin-independent cytotoxin in vivo, protects against *Proteus mirabilis* urinary tract infection. Infect Immun 2009;77(2):632-41. doi:10.1128/iai.01050-08
43. Al-Jubouri SI, Al-Tamimi BJ. Molecular analysis of virulence genes LuxS, RsbA and MRPA in *Proteus mirabilis* Isolated from different clinical samples. Thi-Qar Med J2024;27(1):67-77.
44. Cepas V, Soto SM. Relationship between virulence and resistance among gram-negative bacteria. Antibiotics (Basel) 2020;9(10):719. doi:10.3390/antibiotics9100719
45. World Health Organization (WHO). Antimicrobial Resistance: Global Report on Surveillance. Geneva: WHO; 2014.
46. Garedaghi Y, Shojaee S, Khaki A, Fathiazad F, Khaki AA, Ghdamkheir E, et al. Antiprotozoal effect of *Allium cepa* on acute renal failure caused by *Toxoplasma gondii*. Afr J Pharm Pharmacol 2012;6(10):771-7. doi:10.5897/ajpp12.086
47. Hariri D, Garedaghi Y. Comparison of therapeutic effects of hydroalcoholic extract of asafoetida with metronidazole in mice infected with *Giardia lamblia*. J Zoonotic Dis 2024;8(1):452-9. doi:10.22034/jzd.2024.17396
48. Garedaghi Y, Shojaee S, Khaki A, Hatef A, Ahmadi Ashtiani HR, Rastegar H, et al. Modulating effect of *Allium cepa* on kidney apoptosis caused by *Toxoplasma gondii*. Adv Pharm Bull 2012;2(1):1-6. doi:10.5681/apb.2012.001