

Study of Antibiotic Resistance and the zapA Gene in Proteus Mirabilis Isolated from Patients with Urinary Tract Infections

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Annotation: Background & aim: Proteus mirabilis are Gram negative bacteria belonging to the family of Enterobacteriaceae and have been implicated in a number of infections. In conclusion, the present study was conducted to investigate antibiotic resistance and zapA gene in P. mirabilis from patients with UTI.

Materials & methods: A total of 130 urine samples obtained from patients at Azadi Teaching hospital between March and July 2025 were included in this study. Based on the culturing features of the P. mirabilis colonies developing on MacConkey agar media, the colonies were diagnosed and incubated for 24 hours at 37 °C. the DNA was extracted to detecting ZapA gene for each isolate.

Results: According to the results, 32(24.6%) of the total samples showed positive results for the growth of P. mirabilis when it was cultured on MacConkey agar. Biochemical tests for P. mirabilis bacteria appear to be negative for Indole, oxidase, while, P. mirabilis were positive for urease, citrate, motility, catalase and Kligler iron k/A H₂S. For antibiotic test, P. mirabilis showed a low sensitive 34.4% toward Ceftazidime and a complete resistant 100% toward Ampicillin. Otherwise, P. mirabilis showed high sensitive toward 81.3% Imipenem, 81.3% Levofloxacin, 87.5% Gentamicin, and 90.6% Amikacin respectively. After isolating DNA from P. mirabilis using an extraction and electrophoresis kit, it was found that 100% of P. mirabilis isolates contained the zapA gene. zapA

virulence gene was detected in all 32 *Proteus mirabilis* isolates (100%). Of these, 1 isolate (3.1%) was XDR, 21 isolates (65.6%) were MDR and 10 isolates (31.3%) were sensitive /non-MDR/XDR with there being no significant relationship between the presence of zapA and resistance patterns ($p > 0.05$), suggesting that virulence is not linked to antibiotic resistance.

Conclusions: The results indicate that *P. mirabilis* is an important cause of urinary tract infections and is characterized by a wide range of resistance to antibiotics. On the other hand, it was found that *P. mirabilis* was very sensitive to Amikacin, and the ZapA gene was found in all isolates, which explains the virulence of the bacteria.

Keywords: *P. mirabilis*, UTI, ZapA gene, virulence genes.

Introduction

Urinary tract infections (UTIs) are one of the most frequent infectious diseases, affecting almost 10% of human population over their lifetime [1]. Although many microorganisms, including fungi and viruses, can lead to UTIs, bacteria—accounting for more than 95% of infections—are the most common etiological agents [2]. Urinary tract infection (UTI) is an inflammatory reaction that occurs when pathogenicity sexual organism invade the urinary epithelium. The principle pathogens include the Enterobacteriaceae family of Gram-negative organisms [3]. Of these, *Proteus* species are the second most frequent microorganisms associated with UTIs after *Escherichia coli* *sequelae* [4,5], in particular *Proteus mirabilis*. *Proteus* species are opportunistic Gram-negative bacteria that are ubiquitous in nature, including as normal gut flora of humans and animals. Under optimal conditions, they may induce urinary tract, wound or skin infections [5]. The virulence factors of *P. mirabilis* lead to different opportunistic and nosocomial infections [6,7]. As a pathogenic bacterium it is noteworthy for its involvement in urinary tract infections, gastrointestinal infections, wound infections and bloodstream infection such as catheter-associated UTIs [8]. Toxins, ureolytic enzymes, flagella and adhesins are considered as adequate virulence factors for infection and colonization [9]. *P. mirabilis* strains have also raised antibiotic resistance in recent years, which represents a serious problem to clinicians [10]. These bacteria develop resistance via plasmids, transposons and integrons, resulting in multidrug-resistant (MDR) strains [11]. They use several resistance mechanisms, such as change target sites, antibiotics enzymatic inactivation, efflux pumps and mutagenic rate increment under stress [12]. Many studies have documented worrisome rates of resistance; e.g. [13], 55% of isolates were MDR among *P. mirabilis* strains, Naqid et al. [14], were similarly high in Kurdistan. In hospitals, the resistance rate among *P. mirabilis* strains is competitive with that of *E. coli* (38–48.5%) [15]. However, little data has been reported on the correlation of virulence factor genes such as zapA and antibiotic resistance in *P. mirabilis* isolates in Iraq. Consequently, the purpose of the current study was to investigate antibiotic resistance profiles as well as the prevalence of zapA in *P. mirabilis* strains that were recovered from patients with UTIs.

Materials & methods

Study Design and Sample Collection

This research was carried out from March through to July 2025 in Azadi Teaching Hospital. A number of 130 urine samples from pregnant women in various age groups were obtained. Sterile urine containers were used to prevent contamination and thereby obtain reliable bacteriological results of the midstream urine samples. All specimens were sent to the laboratory for analysis using standard microbiologic techniques.

Isolation and Identification of *Proteus mirabilis*

Bacteriological isolation and identification were conducted by a combined investigative technique using conventional cultural, microscopic, biochemical tests and automated systems of identification.

Characteristics and Microscopic Examination

Culture strength was characterized and observed under microscopical examination. Urine samples were streaked out onto MacConkey agar plates and incubated at 37°C for 24h. Initially, *Proteus mirabilis* was presumptively identified on the basis of its colonial morphology. Gram reaction, morphology of bacterial cell and cellular arrangement were observed under direct microscopic examination by means of Gram staining.

Biochemical Identification

Proteus isolates were identified by a series of routine biochemical tests. Such tests included catalase test and oxidase test, indole production, Methyl Red/Voges-Proskauer (MR-VP) tests, citrate utilization test, urease test, motility culture gelatin liquefaction test and Triple Sugar Iron (TSI) agar.

Identification Automated by VITEK 2 System

Definitive identification of the bacterial isolates was performed with the VITEK® 2 system (bioMérieux). All treatments were conducted as per the manufacturer's recommendations.

Antibiotic Susceptibility Testing

Susceptibility to antibiotics was tested in all isolates according to the disk diffusion method of Kirby-Bauer on Mueller-Hinton agar. Diameters of inhibition zones were determined and interpreted based on the CLSI guidelines (2020).

Statistical Analysis

The distribution and frequency of bacterial isolates and their antibiotic resistance patterns were analysed statistically. Statistical analysis was performed with SPSS software version 26.0. The findings were described as the frequency and percentage of subjects. p-value <0.05 was considered statistically significant.

Molecular Analysis

Genomic DNA Extraction

DNA extraction Genomic DNA was extracted from the isolates with different resistance profile, as recommended by the manufacturer (Geneaid genomic DNA kit, China). DNA concentration and purity were measured on a NanoDrop spectrophotometer (Thermo Fisher Scientific).

PCR Detection of zapA Gene

Polymerase chain reaction (PCR) was used to identify the virulence gene zapA in *P. mirabilis* isolates. The species-specific primers for amplification of the gene are shown in Table 1.

Table (1): zapA gene PCR assayprimers

Primer	Primer sequence	Length (bp)	Ref.
zapA-F	5-ACCGCAGGAAAACATATAGCCC-3	540	[16]
zapA-R	5-GCGACTATCTTTCCGCATAATCA-3		

PCR reactions were carried out in a total volume of 25 μ L including 12.5 μ L of 2 \times Taq PCR PreMix (iNtRON, Korea), 1 AMol/L SO- μ L of the forward and reverse primers each(10pmol/ μ L), 3 μ lof template DNA and7.5 μ L nuclease-free water. Fragmented PCR products were separated on 2% agarose gel and then stained with ethidium bromide visualized under ultraviolet light. PCR products were sequenced by Macrogen DNA Sequencing Service (Korea).

Results and discussion

130 urine samples from patients with UTIs were used in the current investigation (table 2). According to the results, 32(24.6%) of the total samples showed positive results for the growth of *P. mirabilis* when it was cultured on MacConkey agar.

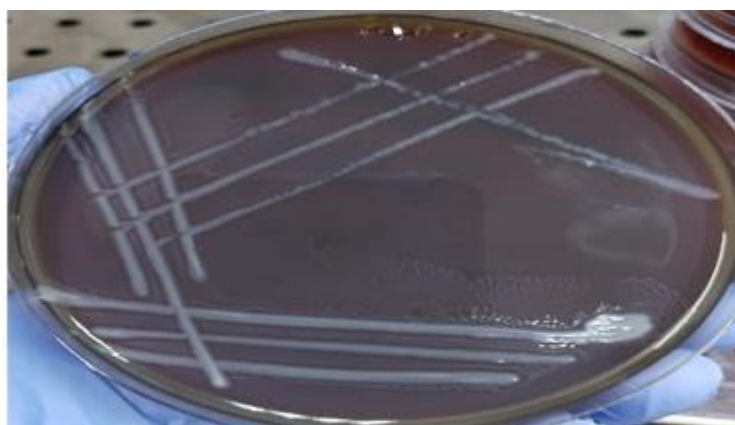
Table (2): Distributed of study samples according to UTI

	No. (%) +ve	No. (%) -ve	Total No.(%)
<i>P. mirabilis</i>	32(24.6%)	98(75.4%)	130(100.0%)

Of 130 samples from different sources of infection, 32 (24.6%) were positive and identified as *Proteus* spp. These findings did not correspond with [17,18] who reported that *Proteus* spp. respectively in the clinical specimens (12.6%), and (8.4%). The higher proportion of local isolated could be due to the broad invasive potential to tissues and implant surfaces via these pathogenic factors by Genus *Proteus*, except for misuse of Antibiotic Drugs which contributes increasing the frequency of infection with *Proteus* strain & Contaminated urinary catheter or other indwelling devices used in unclean environment existed in some hospitals.

Proteus mirabilis identification

Proteus mirabilis are motile gram-negative bacillus. On MacConkey agar (fig: 1), for example, following overnight incubation at 35-37°C, *Proteus* form non-lactose fermenting colonies when individual and the swimming or rippling movement is suppressed by bile salt in the medium, biochemical reaction for *P. mirabilis* bacterial seems to be negative oxidase, Indole but positive catalase urease, citrate, motility and kligler iron k/A H 2 S.

Figure (1): Shows *P. mirabilis* colonies on MacConkey agar

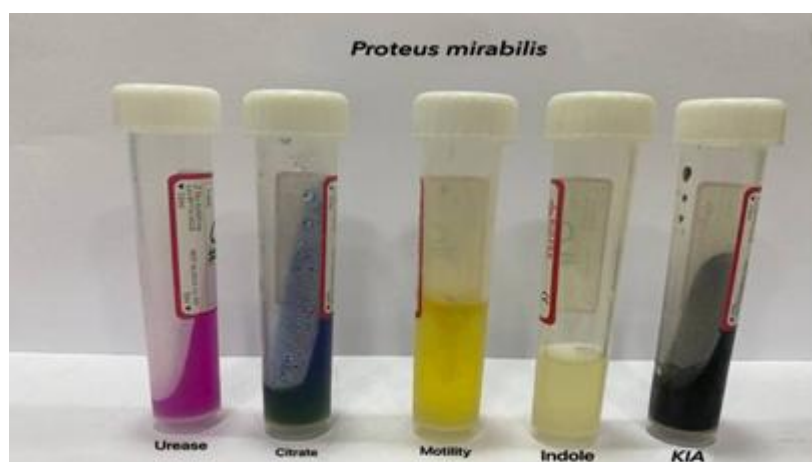


Figure (2): the biochemical tests for *P. mirabilis* isolates.

Antibiotic susceptibility

P. mirabilis showed a low sensitive 34.4% toward Ceftazidime and a complete resistant 100% toward Ampicillin. Otherwise, *P. mirabilis* showed high sensitive toward 81.3% Imipenem, 81.3% Levofloxacin, 87.5% Gentamicin, and 90.6% Amikacin respectively, as shown in table (3).

Table (3): Antibiotic susceptibility test of *P. mirabilis*

Antibiotics	Sensitive %	Intermediate %	Resistant %	P value
Ampicillin	0.0	0.0	100.0	0.001
Nalidixic acid	68.8	0.0	31.2	
Trimethoprim	59.4	3.1	37.5	
Cefotaxime	43.8	3.1	53.1	
CFM	53.1	6.2	40.7	
Gentamicin	87.5	0.0	12.5	
Imipenem	81.3	3.1	15.6	
Amikacin	90.6	6.2	3.1	
Levofloxacin	81.3	0.0	18.7	
Azithromycin	78.1	6.2	14.7	
Ceftazidime	34.4	0.0	65.6	
Ciprofloxacin	56.2	3.1	40.7	

The majority of *P. mirabilis* isolates exhibited limited sensitivity to cefotaxime and ampicillin, respectively. Conversely, gentamycin, azithromycin, imipenem, levofloxacin, and amikacin showed the highest sensitivity. Similar findings have been documented for *P. mirabilis* isolates from Nigeria, where gentamicin resistance rates were 74.1% [19]. The isolates in the Czech Republic displayed resistance rates to gentamicin (25.4%), ampicillin (38.5%), and ciprofloxacin (35.2%), which differ from the results of the current investigation [20]. Different standards and controls for the prescription and administration of antimicrobial drugs, as well as regional variations in the incidence of virulence genes and bacterial strains, may be responsible for the observed disparities in resistance rates. Furthermore, isolates of *P. mirabilis* exhibited great susceptibility to imipenem and moderate to high resistance to antibiotics that prevented the production of cell walls, such as penicillins. The findings of [21], which revealed a high susceptibility to Imipenem in various Enterobacteraceae isolates and moderate to high resistance to some types of first-generation cephalosporins in contrast to low resistance to the majority of third-generation types used in this study despite an increasing rate of resistance to Cefotaxime, supported this. However, these bacteria have the capacity to produce β -lactamases, particularly extended spectrum β -lactamases (ESBLs), as well as to transfer genetic elements containing the

genes of these enzymes. Numerous mutations with these enzymes result in increased resistance to antibiotics, particularly β -lactam, in addition to other mechanisms like altering the target site or altering access to the target site by modification of penicillin binding proteins (PBPs) [22, 23].

zapA gene detection

After isolating DNA from *P. mirabilis* using an extraction and electrophoresis kit, it was found that 100% of *P. mirabilis* isolates contained the zapA gene, as shown in Figure 3.

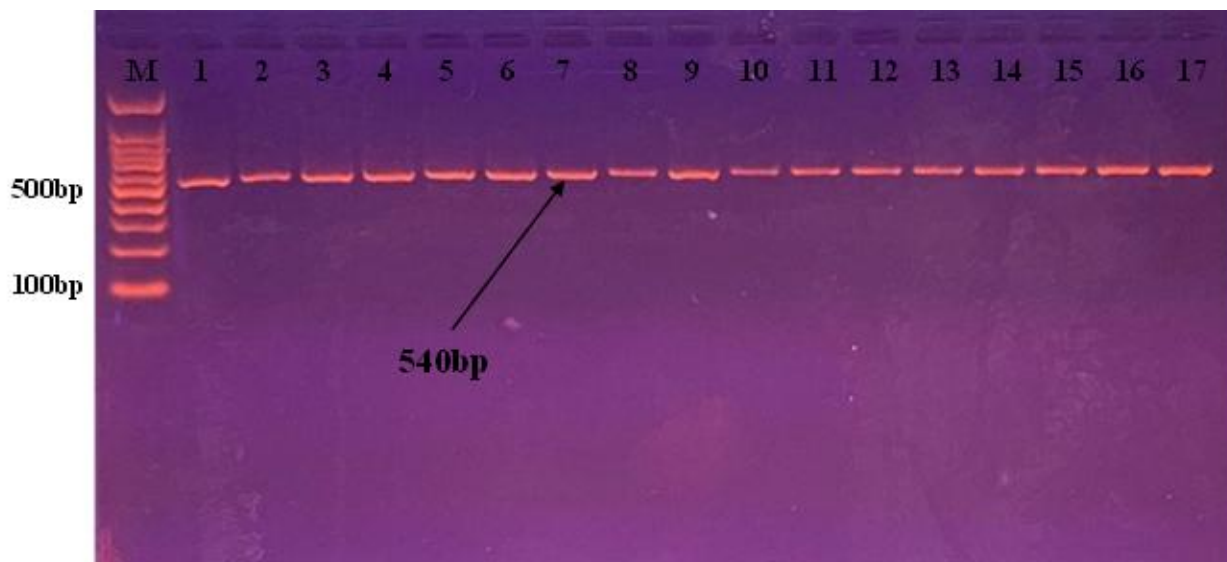


Figure (3): PCR amplification of 540bp zapA gene by 2% agarose gel electrophoresis. Ladder: M, Lane (1-17): PCR product of 17 *P. mirabilis* isolates from urine samples.

P. mirabilis ZapA was found in all 17 isolates, which gave a prevalence of 100%. These genes may be associated with pathogenicity and virulence of the bacterial strains [24]. For example, the *mrpA* gene has been attributed to adhesion, biofilm formation and resistance to host defences [25] and ZapA is a key player in protease activity that mediates local tissue invasion and avoidance of the immune system [26]. Previous evidence studying equivalent bacterial markers in other pathogens have also provided insights into their role during infection and disease. For instance, the association between *mrpA* and ZapA genes in clinical isolates of *P. mirabilis* and their relation with urinary tract infections were also investigated [27]. Although their results support our findings by indicating a wide spread of these genes and linking them to the urinary tract pathogenesis. Likewise, majority of the studies based on *P. mirabilis* and UTI were conducted in Iraq. For instance, a study reported by Alkhalidy and Aburesha [8] found that some virulence genes of *P. mirabilis* from UTI patients in Iraq and stated that these pathogens have virulent factors, which allow them to survive host immunity as well as antibiotics. Alobaidi et al [29] also achieved the detection of ZapA gene in *P. mirabilis* UTI isolates Iraqi patients and emphasized the significance of ZapA gene for bacterial attachment to host tissues. Conclusion: Several pathogenic bacterial isolates were detected in the investigated samples; *P. mirabilis*, *E. coli* and *K. pneumoniae* were the most common UPEC species (respectively) among UTI Iraqi patients with kidney stones. This emphasizes the need for surveillance and monitoring systems to monitor infection rates in hospital settings. The high frequency of the ZapA gene in all *P. mirabilis* isolates tested, suggested that it probably constitutes an important genetic determinant of virulence and pathogenicity. The knowledge of the genetic structure of bacterial pathogens is instrumental in devising therapeutics with specific attributes.

Distribution of zapA Gene and Antibiotic Resistance Classification

All 32 *Proteus mirabilis* isolates (100%) were positive for the zapA virulence gene, indicating that all isolates have pathogenic potential regardless of antibiotic resistance. Upon analyzing resistance patterns: 1 isolate (3.1%) showed extensively drug-resistant (XDR) characteristics, 21

isolates (65.6%) were classified as multi-drug resistant (MDR), and 10 isolates (31.3%) were relatively sensitive or non-MDR/XDR. Despite differences in resistance, all 32 isolates carry the zapA gene, meaning every isolate has the potential to cause disease. Statistical analysis showed no significant difference in the presence of the zapA gene between MDR, XDR, and sensitive isolates ($p > 0.05$), indicating that the virulence gene is independent of antibiotic resistance patterns.

Table 4: Number of Isolates with zapA Gene and Their Resistance Classification

Number of Isolates	Resistance Classification	Percentage of Total (32)	zapA Gene Presence (%)
1	XDR	3.1%	100
21	MDR	65.6%	100
10	Sensitive / Non-MDR/XDR	31.3%	100
Total	—	100%	100

All 32 *P. mirabilis* isolates (100%) were zapA-positive, demonstrating that all of these isolates have a virulence element regardless of their antibiotic resistance. This result is in agreement with studies conducted in Iraq, that highlighted a high prevalence of zapA among clinical and food *P. mirabilis* isolates [30,31]. On the basis of resistance data, 1 (3.1%) isolate was characterized as having XDR pattern, 21 isolates (65.6%) were categorized as MDR and 10 isolates (31.3%) were relatively sensitive or non-MDR/XDR patterns on panel testing. Despite these variations, all isolates were found to harbor the zapA gene and therefore it may be concluded that resistance profile does not have an influence on the presence of this virulence factor [31,32]. The presence of zapA was not statistically different among MDR, XDR and sensitive isolates ($p > 0.05$), as verified by a statistical test [32]. The high percentage of MDR isolates is in accordance with the findings of other Iraqi studies, which have disclosed an increasing trend in antimicrobial resistance among *P. mirabilis*, some isolates achieving XDR categories [33,34]. Crucially, even those isolates that were classified as a sensitive isolate carry zapA gene (therefore have invasive infection potential). This co-occurrence of virulence and resistance represents a major public health challenge and underscores the importance of ongoing monitoring for pathogenic potential and resistance patterns [32,34].

Conclusions

Proteus mirabilis is a significant uropathogen possessing natural pathogenicity associated with the ubiquitous nature of the zapA virulence gene. The virulence seems not to correlate with resistance to antibiotics, it was in fact present both in multi-drug resistant and drug sensitive isolates. Although resistance is widespread, the bacterium is still highly vulnerable to Amikacin and, therefore, of great clinical relevance. These results underscore the importance of ongoing monitoring of not only virulence factors but also resistance profiles for control of *P. mirabilis* urinary tract infection.

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