Original Article

Molecular Study of *blaCTX-M* Profile *among Uropathogenic Proteus mirabilis* Isolates

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Abstracts

Background: *Proteus mirabilis* are the most prevalent uropathogens associated with urinary tract infections (UTIs). Infection occurs only when the pathogenicity of the bacteria surpasses the host's regular defensive systems. A lifetime incidence of between 50% and 60% among adult women, increasing with age. **Materials and Methods:** Collected 450 (325 male and 125 female patients) with UTIs. These patients ranged in age from (17 to 70) years from four Iraqi hospitals (Merjan Hospital, Shomali General Hospital, Al-Hilla Surgical Teaching Hospital, and Babylon Hospital for Maternal and Pediatrics) from the period from July 2021 to the end of October 2022. The identification of bacterial isolates were carried out using standard bacteriological procedures. The antibiotics susceptibility test was carried out by using 12 antibiotics from different classes The molecular identification was done by using polymerase chain reaction amplification of the three bla CTX-M gene (bla CTX-MI, bla CTX-MIV, bla CTX-MII(. **Results:** The percentage of samples positive for *P. mirabilis* based on culture, biochemical, and Gram stain results was 70 (15.5%) of the total 450 samples. The percentage of *P. mirabilis* isolated from male patients was 21 (30%), whereas the percentage of *P. mirabilis* isolated from female patients was 49 (70%) of the total 70 isolates. The result of antibiotic susceptibility showed the high resistance against nitrofurantoin (92.8%), while the higher resistance was against amikacin (10%), imipenem (4.2%), aztreonam (2.8%), and meropenem (1.4%). The result of the current study showed the percentage of bacterial sample positive to the bla CTX-MI, bla CTX-MII, bla CTX-MIV was 9 (12.85), 56 (80), and 40 (57), respectively, among 70 bacteria isolates. **Conclusions:** The result of the study showed the prevalence of antibiotic resistant gene and multidrug resistant among *P. mirabilis* isolated from UTIs.

Keywords: Antibiotics susceptibility, *Proteus mirabilis*, urinary tract infections

Introduction

Over 150 million people worldwide get urinary tract infections (UTIs), which are among the most prevalent bacterial infections.[1] A lifetime incidence of between 50% and 60% among adult women, increasing with age.[2,3] *Proteus* are rod-shaped, gram-negative members of the Enterobacteriaceae family. *Proteus mirabilis*, *Proteus vulgaris*, *Proteus hauseri*, and *Proteus penneri* are the four species of the *Proteus* spp. that are harmful to humans.[4,5]

Proteus mirabilis virulence factors include lipopolysaccharide, flagella, fimbriae, glycocalyx, and the phenomenon of adhesion and hydrophobicity of the bacteria surface, which is a major modification of the bacteria to cause infections.[6]

Gram-negative pathogens that produce extended-spectrum b-lactamase (ESBL) are a major cause of resistance to expanded-spectrum b-lactam antibiotics. They have spread globally since their discovery in the early 1980s. TEM- and SHV-type ESBLs were once the most common ESBL families. Today, CTX-M-type enzymes are the most common ESBL type. ESBL genes are frequently found on plasmids and are housed within transposons or insertion sequences, allowing them to spread.[7,8] The study aimed

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to detected bla CTX-M profile among *P. mirabilis* isolates from UTI patients.

Materials and Methods

Samples collection

A total of 450 mid-stream urine samples were collected from patients suffering UTIs (325 men and 125 women). These patients, who received treatment at three hospitals in Iraq (Merjan Hospital, Shomali General Hospital, Al-Hilla Surgical Teaching Hospital, and Babylon Hospital for Maternal and Pediatrics) between July 2021 and the end of October 2022, varied in age from 17 to 70. All these specimens were transported to laboratory for isolation of bacterial species.[9,10]

Identification of bacteria

The identification of bacterial isolates was carried out using standard bacteriological procedures.[7] All colonies suspected as *Proteus* in primary cultures were purified by subculture on different media. Bacteria were isolated by spreading the samples on blood and MacConkey agar for the first isolation, and then all plates were incubated at 37°C/24 h. Species were identified according to the morphological features on culture media, microscopic examination, and biochemical tests.[11,12] A colony from each bacterial isolate was fixed on a slide and stained by gram stain to examine their reaction and shape according to MacFaddin.[13]

Antibiotics susceptibility test

The antibiotics susceptibility test was carried out by following Kirby Bauer method described by Clinical and Laboratory Standards Institute[14] by using 12 antibiotics from different classes [Ciprofloxacin (5 µg), levofloxacin (5 µg), cefotaxime (30 µg), cefuroxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), nitrofurantoin (300 µg), gentamycin (10 µg), amikacin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg).

Polymerase chain reaction

The ABIO pure extraction method (AVORGEN Biotech Corporation, Taiwan) was used to extract genomic DNA from bacterial growth. The molecular identification was done, by using polymerase chain reaction (PCR) amplification of the [bla CTX-MI (F-5-GACGATGT CAC TGG CTGAGC-3, F-5-GACGATGTCA CTGGCTGAGC-3), bla CTX-MIV (F-5-GCTGGA G AA AAGCAGCGG AG-3, R-5-GTAAGCTGACGC AAC GTC TG-3), bla CTX-MII F-5-GCGACCTGG TTA ACTACA ATC C-3, R-5CGTAG TAT TGCCCT TAAGCC-3] gene.[15] PCR master was mixed and primers' solutions were stirred by vortex, at room temperature. The reaction mixture included 12.5 µL of master mix, 2 µL each of forward and reverse primers, 5 µL of template DNA, and up to 25 µL of nuclease-free water. The components of the tubes were combined using a vortex before being put into the PCR equipment. The PCR condition is shown in Table 1. After PCR amplification, agarose gel electrophoresis was used to establish the amplification's existence. The extracted DNA requirements were totally dependent on PCR.

Ethical approval

The investigation was carried out in conformity with the Declaration of Helsinki's ethical precepts. Before a sample was taken, the patient's verbal and analytical consents were obtained. The study protocol, the subject information, and consent form were reviewed and approved by a local ethics committee with Project No.: M220901 approved at September 28, 2022.

Results

In a cross-sectional study, a total of 450 urine specimens were collected from patients with different ages. The samples included 325 men and 125 women. As selective media, MacConkey agar and Blood agar were employed to examine the distinct colony traits of *Proteus* isolates. As the bile salts are present, *Proteus* isolates produced pale-colored colonies on MacConkey agar medium. *Proteus* was visible as a short rod-shaped, pink, gram-negative organism. Several hemolysis patterns, particularly that of *P. mirabilis*, were seen in *Proteus* isolates on blood agar. These isolates also displayed the swarming phenomena on solid medium [Figure 1].

The percentage of samples positive for *P. mirabilis* based on culture, biochemical, and gram stain results was 70 (15.5%) of the total 450 samples. The percentage of *P. mirabilis* isolated from male patients was 21 (30%), whereas the percentage of *P. mirabilis* isolated from female patients was 49 (70%) of the total 70 isolates [Tables 2 and 3].

The result of antibiotic susceptibility showed the high resistant against Nitrofurantoin (92.8%),

Table 1: PCR thermal cycling conditions of antibiotics resistant genes

Genes	Thermocycling conditions						No. of cycles
	Denaturation		Annealing		Extension		
	Temp. (°C)	Time (s)	Temp (°C)	Time (s)	Temp (°C)	Time (s)	
bla CTX-M-I	94	15	55	30	72	30	30
bla CTX-M-II	94	15	55	30	72	30	30
bla CTX-M-IV	94	30	62	30	72	30	30

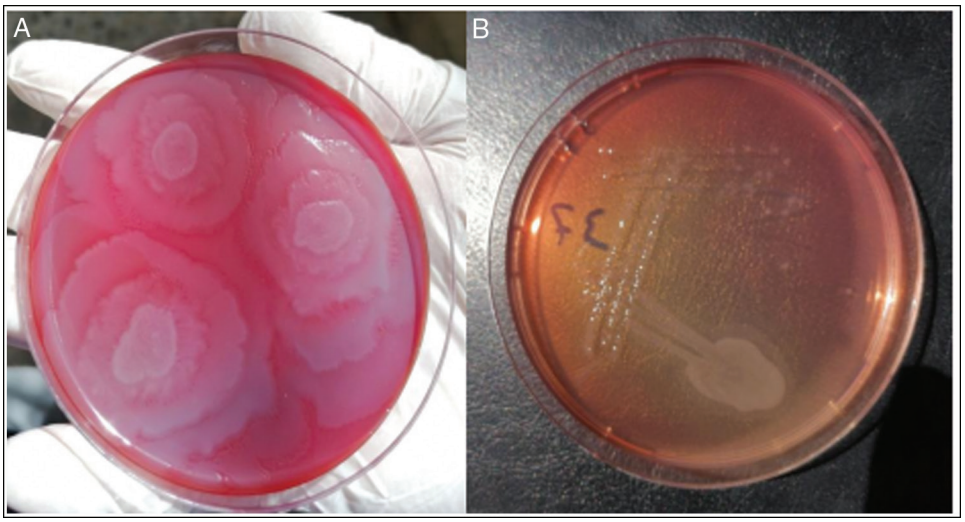

Figure 1: Growth *Proteus mirabilis* on (A) blood agar showing swarming phenomena and (B) MacConky agar showing non-fermenter *Proteus mirabillis*

Table 2: Age groups and gender of patients

Age	Gender		Total No. (%)
	Male No. (%)	Female No. (%)	
<20	23)5.1)	53 (11.7)	76 (16.8)
20–30	45 (10)	161 (35.7)	206 (45.7)
31–40	31 (6.8)	58 (12.8)	89 (19.7)
41–50	11 (2.4)	26 (5.7)	37 (8.2)
51–60	9 (2)	20 (4.4)	29 (6.4)
61–70	6 (1.3)	6 (1.3)	12 (2.6)
>70	0 (0)	1 (0.2)	1 (0.2)
Total	125 (27.7)	325 (72.2)	450 (100%)

Table 3: Number and percentage of *Proteus mirabilis* isolates according to the gender

Gender	Positive samples No (%)	Negative samples No (%)	Total No (%)
Female	49 (10.8)	276 (61.3)	325 (72.2)
Male	21 (4.6)	104)23.1)	125 (27.7)
Total	70 (15.5)	380 (84.4)	450 (100)

Trimethoprim–Sulfamethoxazole (85.7%), Cefotaxime (78.5%), Cefuroxime (68.5%), Ceftazidime (58.5%), Gentamycin (38.5%), Ciprofloxacin (37.1%), Levofloxacin (28.5%). While the lower resistance against Amikacin (10%), Imipenem (4.2%), Aztreonam (2.8%), and Meropenem (1.4%) as shown in Tables 4 and 5.

Genetic detection antibiotic resistant gene

The result of the current study showed the percentage of bacterial sample positive to the bla CTX-MI, bla CTX-MII, bla CTX-MIV was 9 (12.85), 56 (80), 40 (57) respectively among 70 bacteria isolates [Figures 2–4 and Table 6].

Discussion

A total of 450 urine specimens were cultured on a selective medium, MacConkey agar and Blood agar were employed to examine the distinct colony traits of *Proteus* isolates. As the bile salts are present, *Proteus* isolates produced pale-colored colonies on MacConkey agar medium. *Proteus* was visible as a short rod-shaped, pink, gram-negative organism. Several hemolysis patterns, particularly that of *P. mirabilis*, were seen in *Proteus* isolates on blood agar. These isolates also displayed the swarming phenomena on solid medium.

The percentage of samples positive for *P. mirabilis* based on culture, biochemical, and gram stain results was 70 (15.5%) of the total 450 samples.

Proteus species are the most prevalent uropathogens in the general population and hospitals. Infection occurs only when the pathogenicity of the bacteria surpasses the host's regular defensive systems. We have natural defense systems such as urinary tract wall, phagocytic cells, and lymphocytes, as well as nonspecific immunological responses.[13]

Proteus rods are opportunistic bacterial pathogens that cause UTIs, wound infections, neonatal or infantile meningitis, and rheumatoid arthritis when conditions are favorable.[14]

The continuous increase in the antibacterial resistance of clinical bacterial strains has developed a main clinical problem. The rise of several antibiotic resistance mechanisms among prevalent human pathogenic Enterobacteriaceae members raise serious concerns and reduce the number of available treatment options.[15,16]

However, the multidrug-resistant strains of *Proteus* spp. have also been reported worldwide. They have the ability to resist different types of antibiotics, and that is what is called multi antibiotic resistance (MDR).[17,18]

Table 4: Antimicrobial susceptibility test of *Proteus mirabilis* isolates

Family	Antibiotics	Sensitive	Intermediate	Resistance
Quinolones	Ciprofloxacin	6 (8.5%)	38 (52.7%)	26 (37.14%)
	Levofloxacin	25 (35.7%)	25 (35.7%)	20 (28.5%)
Cephems	Cefotaxime	5 (7.14%)	10 (14.2%)	55 (78.5%)
	Cefuroxime	16 (22.8%)	6 (8.5%)	48 (68.5%)
	Ceftazidime	10 (14.28%)	18 (25.7%)	41 (58.5%0
Monobactams	Aztreonam	45 (64.28%)	14 (20%0	11 (2.85%)
Carpenters	Imipenem	45 (64.28%)	22 (31.4%)	3 (4.2%)
	Meropenem	53 (75.7%)	16 (22.85%)	1 (1.4%)
Nitrofurans	Nitrofurantoin	2 (2.85%)	3 (4.28%)	65 (92.8%)
Aminoglycoside	Gentamycin	33 (47.14%)	10 (14.28%)	27 (38.5%)
	Amikacin	57 (81.4%)	6 (8.5%)	7 (10%)
Folate pathway antagonistic	Trimethoprim Sulfamethoxazole	7 (10%)	3 (4.28%)	60 (85.7%)

Table 5: Phenotypic resistance patterns of MDR *Proteus mirabilis* isolates

CLASSES	MDR phenotype	No.	%
3	Cephems/Nitrofurans/Folate pathway antagonistic	15	31.4%
	Quinolones/Nitrofurans/Folate pathway antagonistic	7	
4	Quinolones/Cephems/Nitrofurans/Folate pathway antagonistic	12	38.5%
	Cephems/Monobactams/Nitrofurans/Folate pathway antagonistic	10	
	Quinolones/Monobactams/Nitrofurans/Folate pathway antagonistic	2	
	Cephems/Carpenems/Nitrofurans/Folate pathway antagonistic	3	
5	Quinolones/Cephems/Monobactams/Nitrofurans/Folate pathway antagonistic	3	7.1%
	Quinolones/Cephems/Carpenems/Nitrofurans/Folate pathway antagonistic	2	
6	Quinolones/Cephems/Monobactams/Nitrofurans/Folate pathway antagonistic/Aminoglycoside	4	7.1%
	Quinolones/Cephems/Monobactams/Nitrofurans/Folate pathway antagonistic/Carpenems	1	
7	Quinolones/Cephems/Monobactams/Nitrofurans/Folate pathway antagonistic/Carpenems/Aminoglycoside	1	1.4%
TOTAL		60	85.7

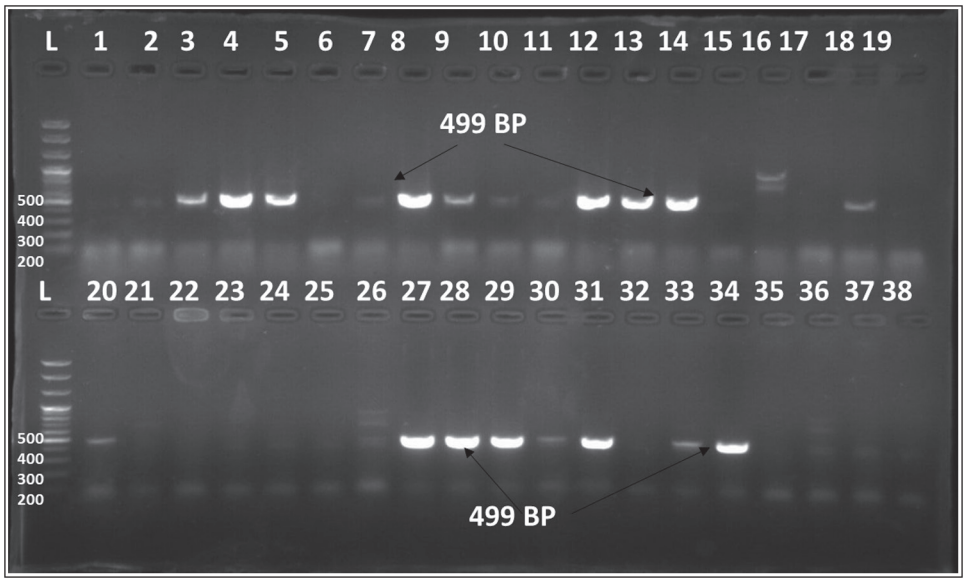

Figure 2: Electrophoresis of 1.5% agarose gel at 70 V for 60 min after staining with red safe for bla CTX-MI (499 bp) PCR products. Lane (L) molecular size marker for DNA molecules (1500 bp ladder), showing positive and negative results

The results of this study concur with the results obtained by Mirzaei *et al*., who found that the bacterial isolates showed a high resistance to amoxicillin with 44.5% and sensitive to imipenem and meropenem in percentage 11.8% and 4.5%, respectively.[18] A total of 150 isolates were tested, and 46 were found to be *P. mirabilis*, with

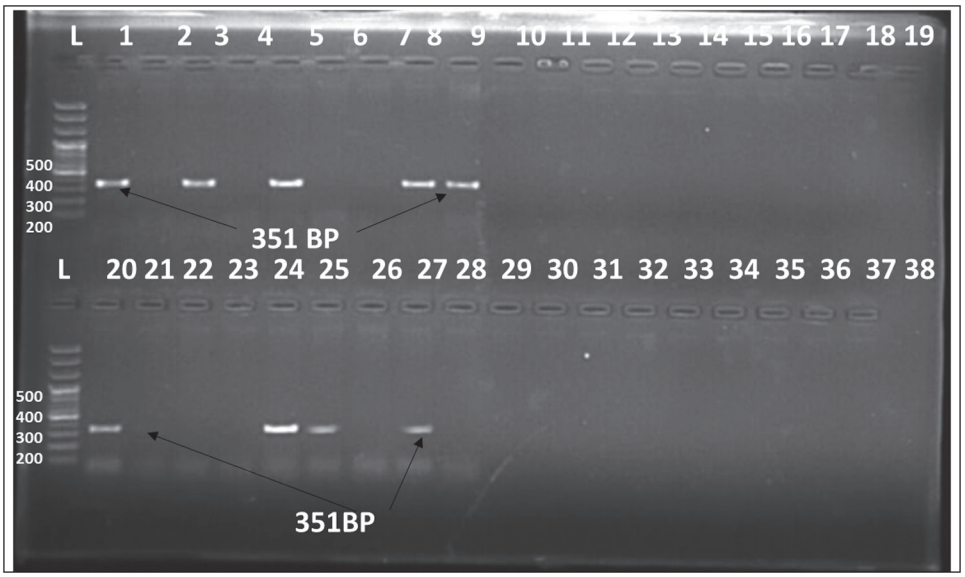

Figure 3: Electrophoresis of 1.5% agarose gel at 70 V for 60 min after staining with red safe for bla CTX-MII (351 bp) PCR products. Lane (L) molecular size marker for DNA molecules (1500 bp ladder), showing positive and negative results

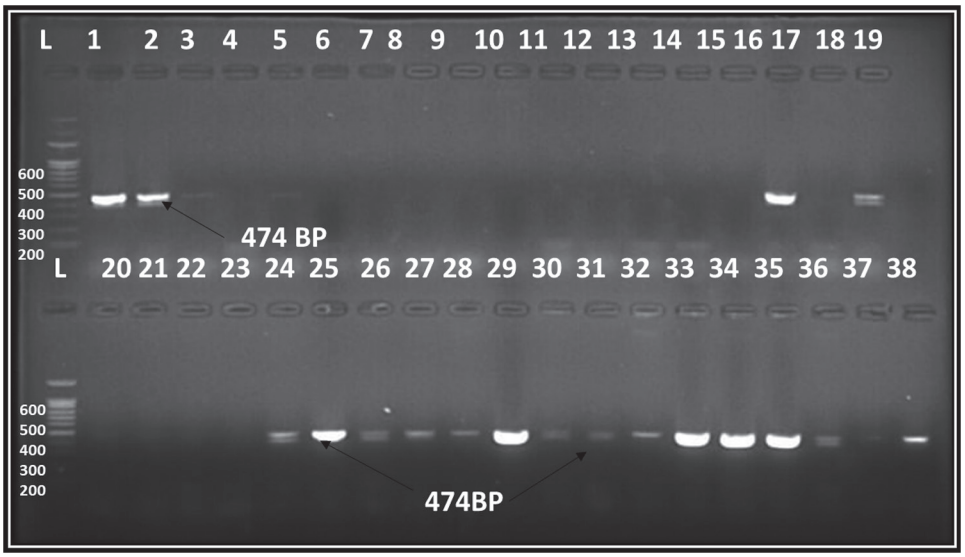

Figure 4: Electrophoresis of 1.5% agarose gel at 70 V for 60 min after staining with red safe for bla CTX-MIV (474 bp) PCR products. Lane (L) molecular size marker for DNA molecules (1500 bp ladder), showing positive and negative results

resistance to levofloxacin and imipenem found to be 60% and 58%, respectively.[19]

According to the current study's findings, 60 of the 70 bacteria isolates (or 85.7%) were MDR-positive. Comparative to sensitive strains, which primarily exhibited incompatible growth with aggressive swarming motion and weaker biofilm, multidrug-resistant *P. mirabilis* strains more frequently exhibited mutual growth between each other, weaker swarming motility, and stronger biofilm.[20] Multidrug-resistant *P. mirabilis* strains more often exhibited mutual growth between each other, weaker swarming motility, and stronger biofilm in comparison with sensitive strains, which represented mostly incompatible growth with aggressive swarming motility and weaker biofilm.[20]

The clinical microbiology laboratory can use both phenotypic and genotypic testing to identify b-lactamases, among other diagnostic methods. Globally, ESBL-producing gram-negative organisms will continue to be a major cause of antibiotic resistance. It is crucial that we continue to be watchful in spotting them in patient isolates as well as through monitoring investigations.[8]

Enzymes of the CTX-M type b-lactamase were first discovered in the late 1980s and simultaneously appeared in numerous sites. A study from Germany is where

Table 6: Number and percentage antibiotic resistant gene among *Proteus mirabilis* isolates

Gene	Positive samples No (%)
bla CTX-MI	9 (12.85)
bla CTX-MII	56 (80)
bla CTX-MIV	40 (57)

the name CTX-M (cefotaximase from Munich) first appeared.[21]

In several countries, epidemics occurred after these initial findings. The "CTX-M pandemic" is the term used to describe the global spread of isolates bearing these ESBLs.[22-24]

Considering the sequence homologies, the majority of CTX-M enzymes may be divided into five groups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. The CTX-M15 group is by far the most prevalent CTX-M-1 subgroup, followed by CTX-M-3 and CTX-M-1.44. The most prevalent enzymes in the CTX-M-9 group were CTX-M-9 and CTX-M-14, although more recently, CTX-M-27 has been recorded often. Within their respective groups, CTX-M-2, CTX-M-8, and CTX-M-25 are the most prevalent varieties.[25-27]

Early CTX-M enzymes exhibited little ability to break down ceftazidime, in contrast to TEM- and SHV-type ESBLs that had been previously characterized. However, later studies described CTX-M variations with increased ability to break down ceftazidime. Notable CTX-M enzymes from the CTX-M-1 and CTX-M-9 families that exhibit ceftazidime hydrolysis are CTX-M-15 and CTX-M-27. When compared to CTX-M-3, which it is descended from, CTX-M-15 has one amino acid changed at position 240 (from Asp to Gly).[28,29]

Conclusion

The result of the study showed the prevalence of antibiotic resistant gene and multidrug resistant among *P. mirabilis* isolated from UTIs.

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Conflicts of interest

There are no conflicts of interest.

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